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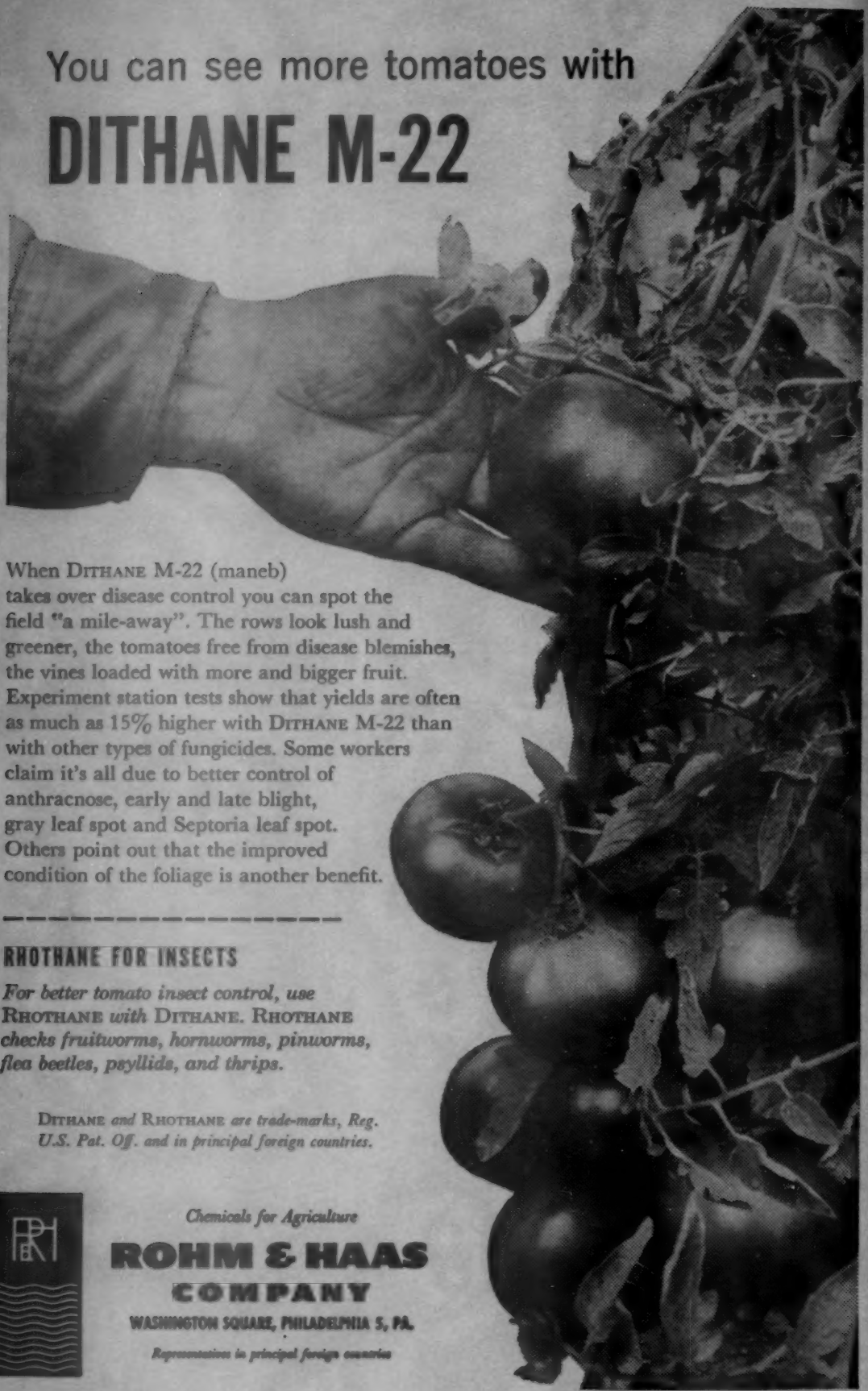

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DIFFERENTIAL TRANSMISSION OF PEACH YELLOW LEAF ROLL VIRUS TO PEACH AND CELERY BY THE LEAFHOPPER, COLLADONUS MONTANUS[1]

D. D. Jensen

SUMMARY

The leafhopper, *Colladonus montanus* (Van Duzee), was shown to vary markedly in its ability to transmit peach yellow leaf roll virus, depending upon the test plant used. When peach was used as both the virus source and test plant, transmission was accomplished to only 1 of 168 peach trees. In tests with celery as both inoculum and test plant, *C. montanus* transmitted virus to 232 of 546 celery plants. When tested singly, 15 of 20 leafhoppers proved to be viruliferous. In successive transfers, these 15 leafhoppers transmitted virus to 43 of 123 celery plants. The test feeding time was 1 day on most plants. Even when demonstrably viruliferous, *C. montanus* proved to be an inefficient vector on peach. After acquiring virus from diseased celery plants, 38 groups, each consisting of 4–10 leafhoppers, were fed alternately on peach and celery for 24-hour periods. These leafhoppers transmitted virus to 57 of 213 celery plants but to only 4 of 238 peach trees. Although *C. montanus* is numerically the dominant species among the known insect vectors of peach yellow leaf roll virus in the Sacramento Valley of California, it is suggested that this species is of less economic importance than is *C. geminatus*.

The geminate leafhopper, *Colladonus geminatus* (Van Duzee), was the first insect to be reported as a vector of viruses in the complex usually referred to as western X-disease of stone fruits (6, 9). It has also been considered the most important economic vector of viruses in this complex—particularly in the Pacific Northwest where it may be found in large numbers in the fruit-growing regions.

Closely related to *C. geminatus* is the species *C. montanus* (Van Duzee), which Wolfe (8) reported as a vector of western X-disease virus. These 2 species have the same geographic range, complete their life cycle on a wide variety of plants (20 species of weeds being hosts common to both leafhoppers (1)), and have been shown to transmit the California strain of aster yellows virus (7). These biological similarities made *C. montanus* a logical suspect as a vector of the stone fruit virus strains transmitted by *C. geminatus*. An understanding of the vector role of *C. montanus* is of particular importance in California where the species is approximately 10 times as abundant as is *C. geminatus* in Yuba and Sutter Counties, which constitute the important "peach bowl" of the state.

Early attempts to transmit peach yellow leaf roll virus by means of *C. montanus* were unsuccessful. A possible explanation for this failure was the high mortality of *C. montanus* when confined to peach in contrast to the long survival of *C. geminatus* on this plant (3). This paper also indicated that more suitable conditions for survival of *C. montanus* and for virus transmission could be provided by giving the leafhopper feeding periods of 2 or 3 days on peach, alternated with similar periods on a more compatible host such as celery. By this method, both longer acquisition and transmission feeding periods could be achieved. The present paper reports transmission of peach yellow leaf roll virus to both peach and celery by means of *C. montanus* and shows that this leafhopper, even when demonstrably viruliferous, is an exceedingly inefficient vector on peach.

MATERIALS AND METHODS.—All vector tests were conducted in the greenhouse at Berkeley, California. *Colladonus montanus* was reared on celery in the greenhouse. *Colladonus geminatus*, collected in the field or reared in the greenhouse, was used for the transmission of virus from peach to the first celery plants from which *C. montanus* recovered virus. The virus source plants were Lovell peach seedlings grown in the greenhouse and inoculated by means of *C. geminatus*, and celery plants, *Apium graveolens*, var. Golden Self Blanching and Utah Green Jumbo, inoculated by means of *C. geminatus* or *C. montanus*. The test plants grown in the greenhouse were Lovell peach seedlings and celery plants of the 2 varieties listed above. When fed upon by leafhoppers, test plants were growing in 4- or 6-in. pots and varied in height from 6 to 15 in. A large number of peach and celery control plants remained healthy throughout the experiments.

RESULTS.—*Field abundance of leafhoppers.*—The abundance of *Colladonus montanus* and *C. geminatus* varies from region to region, as does the ratio of the 2 species. In the Pacific Northwest, *C. geminatus* appears to be the dominant species and at times occurs in very high numbers. In California, the 2 species vary in number from 1 area to another. At Green Valley, Solano County, no specimens of *C. montanus* were collected on 12 yellow sticky boards maintained in a cherry orchard from May 10 to July 1, 1956; however, a total of 1–5 specimens of *C. geminatus* were caught during each 7- to 14-day exposure throughout this sampling season. At San Leandro, Alameda County, both species can usually be collected in rather high numbers in delphinium plantings to which insecticides have not been applied.

In the peach area of Yuba and Sutter Counties, the population of both species is relatively low in most years, with *C. montanus* being consistently more abundant than *C. geminatus* (Table 1). Since 1951, the leafhopper fauna of this area has been sampled at

[1] Accepted for publication April 27, 1957.

TABLE 1.—*Abundance of Colladonus montanus and C. geminatus in 6 alfalfa fields of Yuba and Sutter Counties in 1953 and 1954*

Month	Average number leafhoppers per 1000 sweeps			
	1953		1954	
	C. montanus	*C. geminatus*	*C. montanus*	*C. geminatus*
April	9.0	0.0	0.0	0.0
May	17.5	0.0	41.0	4.1
June	10.0	1.9		
July	10.0	1.9	20.0	0.6
August	—	—	1.5	0.0
September	14.8	2.2	0.0	0.0
October	14.0	1.8	—	—
November	9.0	0.0	—	—
December	1.5	0.0	—	—

TABLE 2.—*Transmission of peach yellow leaf roll virus from peach to peach by means of Colladonus montanus*

Life stage on inoculum	Life stage on test trees	Days on inoculum	Leafhoppers per test tree	Results
Adults	Adults	4	5	0/18 [a]
Adults	Adults	11 [b]	1	0/24
Adults	Adults	14 [b]	1	0/10
Adults	Adults	18 [b]	15 [c]	0/8
Nymphs	Adults	2	1	0/106
Nymphs [d]	Nymphs	20-30	15	1/2
			Totals	1/168

[a] Denominator is the number of plants fed upon; numerator is the number of plants that became infected.
[b] Time on inoculum was discontinuous, with 2–3 days on diseased peach alternated with 2–3 days on healthy celery.
[c] Time on test trees was discontinuous, with 2–3 days on peach alternated with 2–3 days on healthy celery.
[d] Nymphs hatched on diseased peach and remained on inoculum continuously for 20–30 days.

intervals to determine the abundance of known and potential vectors. Collections, taken by means of a sweep net, were made from fruit trees, orchard cover crops, pastures, field crops, and uncultivated herbaceous and woody plants in the area. Alfalfa fields were consistently the most productive sources for both species.

During 1951, regular samples were not taken, but both *C. montanus* and *C. geminatus* could be collected on alfalfa without difficulty. In 1952, the population of both species was very low. Samples were taken from 6 locations on 1 day of each of the following months: May, June, July, August, and October. During the season, only 24 specimens of *C. montanus* and 3 of *C. geminatus* were taken. The sampling data for 1953 and 1954 are presented in Table 1.

C. montanus was approximately 10 times more abundant than was *C. geminatus* in the peach district in which yellow leaf roll virus of peach occurs. This suggested that *C. montanus* might be a more important economic vector than is *C. geminatus* unless its ability to acquire and/or transmit the virus should prove so low as to nullify its numerical superiority.

Transmission from peach to peach 1951-1953.—Prior to the discovery of celery as a host plant of peach yellow leaf roll virus and related strains (4, 5), *C. montanus* had been tested as a possible vector; peach was used as the virus source as well as the test plant (Table 2). This leafhopper, when reared on celery, usually survives only a few days when transferred to peach (3); however, the adults will lay eggs in the peach leaves, and the hatching nymphs may survive on this host for several weeks but usually fail to reach the adult stage.

In some of the tests, the total feeding time on both diseased and healthy peach trees was extended by feeding the leafhoppers alternately on peach and celery for 2–3 days. By this means, acquisition feeding times as high as 18 days on peach were achieved with leafhoppers that had been reared on celery. After feeding on diseased trees, adult leafhoppers in most tests were held on celery plants for several weeks before transfer to healthy peach trees. This was to permit expiration of the incubation period of the virus in the leafhopper, which had been shown to be between 30 and 45 days in most individuals of *C. geminatus*. A similarly long incubation period was assumed for *C. montanus*. In other tests, eggs were laid in peach leaves during 2- to 4-day confinements on diseased trees. These eggs hatched, and the nymphs were left on the inoculum trees for 20–30 days. Survivors were then transferred to healthy peach trees for the transmission feedings. Disease symptoms developed in 1 test peach tree fed on by such nymphs. This was the only case of transmission from peach to peach by means of *C. montanus* in 168 tests. It should be pointed out, however, that 140 of these tests were made with single insects.

Wolfe (8) reported similar difficulty in demonstrating *C. montanus* as a vector of western X-disease virus in Washington, but he obtained 4 cases of transmission in 97 tests.

Transmission from celery to celery.—The poor survival of *C. montanus* on peach made it difficult to conduct extensive tests with this leafhopper. The reason for its apparent low vector efficiency was not known, but high mortality on peach seemed to be 1 probable cause. Apart from this, however, *C. montanus* appeared to be inherently inferior to *C. geminatus* as a vector of peach yellow leaf roll virus from peach to peach, the latter insect species having been demonstrated to transmit this virus to and from peach with considerably greater efficiency (2, 5).

The discovery that celery is susceptible to viruses of the western X-disease complex (4, 5) made it possible to study more effectively the role of *C. montanus* as a vector of these viruses. This leafhopper breeds readily and survives for long periods on celery. Moreover, previous work (5) had indicated that celery is a better virus source for *C. geminatus* than is peach. During 1955, when celery was used as the virus source plant, *C. montanus* transmitted peach yellow leaf roll virus to 18 of 21 celery plants. The acquisition feeding time on the inoculum source was 6–9 days during the nymphal stage. In 15 of these tests, the number of insects per

TABLE 3.—*Serial transmission of peach yellow leaf roll virus from celery to celery by 15 single adults of Colladonus montanus* [a]

Time on consecutive test plants (days)	Transmissions [b] by indicated insect															Totals
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1	+	−	+	+	−	−	−	+	+	+	−	−	−	−	+	7/15
1	−	−	−	−	+	+	−	+	−	+	+	−	+	+	−	7/15
1	−	−	−	+	−	−	−	+	+	+	−	−	−	−	−	4/15
4	−	+	+	+	+	+	+	+	−	+	+	+	−	+	+	12/15
1	−	+	+	−	−	+	−	+	(D)	(D)	−	−	−	(D)	−	4/12
1	−	−	−	(D)	+	−	(D)	+			−	−	−		−	2/10
1	−	−	+		−	−		−			−	+	−		−	2/10
1	−	−	−		+	−		−			−	+	−		−	2/10
3	(D)	−	−		−	+		−			−	+	−		+	3/9
3		−	−		−	−		(D)			−	(D)	−[d]		−	0/7
		(D)	(D)		(D)	(D)					(D)				(D)	
Totals [c]	1/8	2/10	4/10	3/5	4/10	4/10	1/5	6/9	2/4	4/4	2/10	4/9	1/16	2/4	3/9	43/123

[a] Five additional individuals failed to transmit the virus before dying. Feeding time on inoculum was 7 days, after which the insects were held for 41 days before starting test feedings.
[b] + Indicates that plant became infected, — that it did not, and (D) that insect died on preceding plant.
[c] Denominator is the number of plants fed upon; numerator is the number of plants that became infected.
[d] Leafhopper No. 13 survived for 7 additional days during which it fed on 6 plants; no further infections resulted.

test group was between 3 and 10. Greater numbers were used in the other 6 tests.

During 1956, more extensive tests were conducted with celery used as the virus source. In some of these experiments, celery and peach were alternated as test plants; in others, only celery was used. Groups of adults of *C. montanus* transmitted the virus to 38 of 48 celery plants in miscellaneous tests not included in Tables 3 and 4. In this series, nymphs had been allowed an acquisition feeding period of 4–6 days on diseased celery. They were then held on healthy celery for approximately 30 days before the test feedings, most of which lasted 4 days. Of the 38 positive tests, 31 were with groups of 4–15 leafhoppers and 7 were with groups of 37–100 leafhoppers. Eight of the 10 failures were with groups of 3–6 insects feeding 2 days, and 2 were with groups of 9 feeding 4 days.

Serial transmission by single insects.—In an experiment designed to determine the ability of individuals of *C. montanus* to transmit both peach yellow leaf roll virus and aster yellows virus, single insects were tested successively on healthy celery plants. During the nymphal stage, these insects had fed for 7 days on celery infected with yellow leaf roll virus after which they were held on healthy celery for 21 days. They were then caged for 20 days on celery infected with the California strain of aster yellows virus. Thereafter, 20 leafhoppers were caged singly on young celery plants for serial transfers (Table 3).

Of the 20 leafhoppers, 15 transmitted peach yellow leaf roll virus to 1 or more celery plants. Because of contamination of test plants with insecticide, only 1 viruliferous leafhopper survived longer than 2 weeks on the test plants; 1 of the 16 plants it fed upon became infected. Of 123 plants fed on in this experiment, 43 became infected. One individual transmitted virus to all 4 of the plants it fed upon whereas another insect transmitted to the first 6 of 9 plants fed upon. Transmission by the other individuals was more random in pattern.

None of the insects listed in Table 3 transmitted aster yellows virus; however, no conclusions can be drawn from this experiment regarding transmission of aster yellows virus in the presence of peach yellow leaf roll virus. *C. montanus* is an inefficient vector of aster yellows virus to celery, and the test insects died before sufficient time had elapsed to insure completion of the incubation period of the virus in the leafhoppers.

Transmission during alternating 24-hour periods on celery and peach.—Failure of *C. montanus* to transmit virus from stone fruit to stone fruit, except rarely, could be due to the unsuitability of peach as a virus source, as a test plant, or both. The discovery of celery as an effective virus source and test plant for *C. montanus* provided opportunity for more precise evaluation of this leafhopper species as a potentially dangerous vector in peach orchards.

An experiment was designed to compare the transmitting ability of *C. montanus* on peach and on celery by the same insects during alternating feeding periods (Table 4). Virus-free nymphs were caged on 5 differ-

TABLE 4.—*Transmission of peach yellow leaf roll virus from celery by Colladonus montanus, in groups of 4–10 leafhoppers, during alternating 24-hour periods on celery and peach and in 2- to 4-day periods on celery*

Number of groups	Days on inoculum	Transmissions [a] during indicated test feeding period on test plant indicated		
		1 Day		2-4 Days
		Celery	Peach	Celery
11	4	17/60	0/65	20/35
10	4	15/56	3/60	19/34
7	4	9/31	1/44	18/29
4	6	4/17	0/17	8/11
6	5	12/49	0/52	8/29
Totals 38		57/213	4/238	73/138

[a] Denominator is the number of plants fed upon; numerator is the number of plants that became infected.

ent diseased celery plants for acquisition feeding periods of 4–6 days. They were then held on healthy celery plants for 33–43 days before serial transfers to the test plants.

In the 5 series of tests, summarized in Table 4, 38 different groups of leafhoppers were fed for alternating 24-hour periods on peach and celery. The number of different peach and celery plants fed on by a single group ranged from 4 to 26, with a mean of 6.3 peach trees and 5.6 celery plants. Groups of 10 leafhoppers were used; the survivors were counted at each transfer and were not used in groups consisting of fewer than 4 insects. Smaller groups were combined to provide 4 or more individuals per test feeding. Because of a shortage of celery plants, the groups could not be transferred daily throughout the experiment; therefore, they were held on separate celery plants for 2- to 4-day periods on weekends.

Table 4 shows that 38 groups transmitted virus to 57 of the 213 celery plants but to only 4 of 238 peach trees upon which they were caged for 24-hour periods. The same groups transmitted virus to 73 of 138 celery plants exposed for 2- to 4-day periods.

In a separate experiment, a group of 60 insects (*C. montanus*) transmitted virus to a single peach tree and to a celery plant, each exposed to feeding for 24 hours. Another group of 100 leafhoppers failed to transmit virus to a peach tree during a 24-hour period but did transmit it to 2 celery plants fed on before and after the peach feeding.

Symptoms on green-type celery.—Symptoms caused by peach yellow leaf roll virus and related strains in Golden Self Blanching variety of celery were described earlier (5). The disease is apparent in 4–6 weeks, and the plants usually wilt and die within 2 months after inoculation.

The Utah Green Jumbo variety of celery develops symptoms a little more slowly than does Golden Self Blanching, and infected plants survive as long as 4 months. About 5 weeks after inoculation, the terminal leaflets of the largest leaves become lighter green in color than normal and develop upward cupping or rolling. Within another week, the color is almost lemon yellow, rolling is more pronounced, and the leaves are stiffer than normal. Thereafter, the youngest leaves make little growth, remaining dwarfed, chlorotic, and usually malformed. Green color may persist for several weeks in the partially expanded leaves, but the largest leaves develop chlorosis and the interveinal tissue becomes blanched. Ultimately, all infected plants die, but they decline gradually and survive for weeks after developing conspicuous symptoms. This is in contrast to the Golden Self Blanching variety in which the virus causes a collapse of the root system and death of the plant within 2–3 weeks after foliage symptoms become evident.

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A VANILLA HYBRID RESISTANT TO FUSARIUM ROOT ROT[1]

Thomas Theis and F. A. Jiménez

SUMMARY

The cultivation of vanilla is seriously hampered by a root-rot disease caused by *Fusarium batatatis* var. *vanillae*. Attempts to develop high-quality, disease-resistant plants have been made by crossing *Vanilla planifolia* and *V. phaeantha*. The latter has small fruits of poor quality but is resistant to root rot. Some of the resulting hybrids grew poorly, and such plants were found to be severely affected by root rot. Twenty-six plants were large and vigorous, and these plants had good root systems. Growth characters of the hybrids were studied to determine if some readily apparent trait was associated with disease reaction, but none was found. The flowers of the hybrids are intermediate in size between the 2 parents. Variations of leaf shape occurred that ranged from leaves typical of 1 parent to those of the other. The hybrid pods were intermediate in size. The quality of extract from the hybrid fruits is not equivalent to the high-grade products obtained from *V. planifolia*, but it is similar to the less valuable ones obtained from *V. tahitensis*. The hybrids commenced flowering when the plants were 8 years old. *V. phaeantha* flowers sparsely, and its fruits drop before maturity. *V. planifolia*, in contrast, can be relied on to produce an adequate supply of flowers and to retain its fruits to maturity. The hybrids flowered erratically and fruit retention was not dependable. Further backcrossing to *V. planifolia* is necessary, not only to improve fruit quality, but also to increase the amount of flowering and to improve fruit set and retention.

The cultivation of *Vanilla planifolia* Andrews, the source of vanilla flavoring, is seriously hampered wherever it is grown by a root rot disease caused by *Fusarium batatatis* var. *vanillae* Tucker (1, 3, 10, 12). Attempts to develop high-quality, disease-resistant plants by breeding have been made (9, 11) by crossing *V. planifolia* with other disease-resistant species of vanilla. In these early trials, pollination of *V. planifolia* was successful and fruit and seeds were formed, but hybrid plants were not obtained because the seeds failed to germinate. Vanilla seeds are capable of germination, and reports of this have been reviewed by Bouriquet (2); nevertheless, few plants had been obtained in this manner until 1950.

The development of vanilla hybrids that combine quality and disease resistance was made possible in 1950 by Knudson's (8) studies on germination of vanilla seeds. He obtained plants from seeds of a cross of *Vanilla planifolia* × *V. phaeantha* Reich. made at this station. The latter species has small fruits of poor quality but is reported (7) to be resistant to root rot. The hybrid seedlings were returned (4) to Puerto Rico and eventually were established in the field (6). The purpose of the present paper is to report the disease reaction, growth characteristics, fruit quality, and flowering and fruiting habits of these vanilla hybrids.

METHODS AND MATERIALS.—Forty hybrid seedlings were received in January, 1947. They were planted in pots containing a mixture of peat moss and expanded mica and were watered regularly; in addition, 20 ml of of a WP (13) nutrient solution was given to each plant weekly. Three seedlings died during the first year while the plants were in the greenhouse.

The remaining plants were transplanted to the field at a site known to be infested with the root rot organism: in previous preliminary trials, all plants of *Vanilla fragrans* in this area became diseased. The hybrids were planted at 3-ft. intervals in beds in a bamboo lath house that permitted entrance of about 50 per cent sunlight. The beds were 2 × 20 ft. and consisted of a 3-in. layer of coarse river gravel covered with a 2-ft. mulch composed of a mixture of cut native grasses. Bucare trees (*Erythrina berteroana* Urban) spaced at 2-ft. intervals supported the vines. Nutrient solution was applied to the hybrids until the plants were 8 years old.

RESULTS.—*Disease reaction of mature plants.*—Differences in growth among the seedlings were apparent soon after they were transplanted to the field. Some of the hybrids grew poorly, and their leaves were light green and flaccid. Such plants were badly infected with the root rot organism. Six plants have failed to survive because of this disease, and at the present time, 5 others are badly infected but still living. Twenty-six plants are large and vigorous and have good root systems (Fig. 1).

Comparison of growth characters of hybrids and parents.—Growth characters of the hybrids were studied to determine if some readily apparent trait was associated with disease reaction. The general plant color, internode length, and the leaf type were recorded. Leaf color is darker and the internode length is longer in *V. phaeantha* than in *V. planifolia*. Seven hybrid plants were similar to *V. planifolia*, and 4 were like *V. phaeantha*; the remaining plants were intermediate or mixed for 1 or more traits. Badly diseased plants occurred among the 3 groups, and no character appeared to be correlated with disease resistance or susceptibility.

The length of the sepals, petals, and lips of the flowers from parent species and hybrids was measured, since the sizes of the floral parts of *V. planifolia* and *V. phaeantha* are different (5). Fifteen flowers were selected at random from each of the parent species and the hybrids. Measurements of some of the floral structures are given in Table 1. These data show that

[1] Accepted for publication April 24, 1957.

FIG. 1. The root development of a hybrid vanilla from a cross of *Vanilla planifolia* × *V. phaeantha*.

the flowers of the hybrids are intermediate in size.

The leaf types of the hybrids and parents were compared, since these traits are different in the parent species; in fact, they are so pronounced that they have been used in nomenclature (5). The leaves of *V. planifolia* are relatively large; some as long as 23 cm were observed. They are somewhat broad in comparison to length; the mean width of 25 leaves 13–15 cm long was 4.3 cm, and the range was 4.2 to 4.5 cm. The mean width of leaves of *V. phaeantha* 14–16 cm long was 3.6 cm and the range was 2.9 to 4.0 cm. The longest leaf of *V. phaeantha* observed was 18 cm. Among the hybrids, variations of leaf shape on different plants ranged from leaves typical of 1 parent to those of the other (Fig. 2).

The fruit length of the hybrids was determined, since this has a bearing on value. The fruits of *V. phaeantha* are undesirable commercially because they not only have poor quality but their length, which is about 9 cm, does not meet trade requirements. Fruits of *V. planifolia*, in contrast, have good quality and

TABLE 1.—*A comparison of the size of floral structures of a vanilla hybrid (V. planifolia × V. phaeantha) with those of the parent species*

Flower structure	*V. planifolia*		*V. phaeantha*		Hybrid	
	Mean[a]	Range	Mean[a]	Range	Mean[a]	Range
	cm	*cm*	*cm*	*cm*	*cm*	*cm*
Sepal length	5.8	5.4-6.0	8.1	7.5-9.0	7.1	7.0-7.5
Petal length	5.7	5.3-6.1	8.0	8.0-8.7	7.1	7.0-7.5
Lip length	4.8	4.3-5.0	7.8	7.5-8.3	6.0	6.0-6.5

[a] From measurements of 15 flowers.

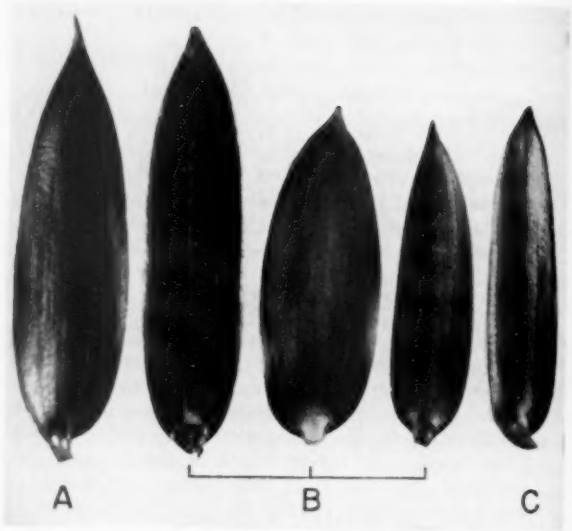

FIG. 2. Typical leaves of *Vanilla planifolia* (A) and *V. phaeantha* (C) and of different selections of hybrids between these 2 species (B).

their average length of 18 cm is satisfactory. The hybrid pods (Fig. 3) were intermediate in size. The mean length of 26 pods from 4 vines was 13.9 cm, with a range of 10.5 to 17.5 cm. Although the hybrid pods are not as long as desired, they might be accepted if quality were good.

Fruit quality determinations.—The quality of extract from the hybrid fruits was determined.[2] The analytical report was as follows: lead number, 0.727 on 5 ml; vanillin, 0.18 g in 100 ml. This lead number indicates

[2] By David Michael & Co., Vanilla Products, 3743 D Street, Philadelphia 24, Pennsylvania.

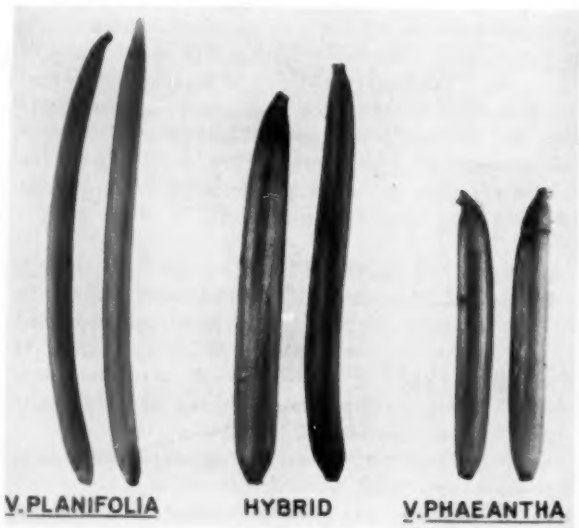

FIG. 3. A comparison of size of fruit from the vanilla hybrid *Vanilla planifolia* × *V. phaeantha* with those of the parents.

an extract of normal strength, but the vanillin content was somewhat lower than in extracts of *V. planifolia*. The color of the extract from the hybrids was excellent. The aroma was decidedly cinnamic, which detracts from quality. Though the extract from the hybrids is not equivalent to the high-grade products obtained from *V. planifolia*, it is similar to the less valuable ones obtained from a different species of vanilla, *V. tahitensis* J. W. Moore.

Flowering and fruiting characters.—The hybrids commenced flowering in 1951, when the plants were 8 years old. A record of flowering of the individual plants was taken (Table 2). Flowering of the hybrids during the 1955 season was relatively abundant. Ten plants bore flowers during this season, as compared to 1 plant in 1954 and 7 in 1952. The plants with inflorescences in 1955 included some that had never borne before. More notable, however, is that some plants that flowered previously failed to flower this year. Flowering was recorded for hybrid No. 20, for example, during 1951 and 1954 but not in 1955. Hybrids No. 31 and No. 32 flowered in 1952 but not in 1954 and 1955.

A new planting of the hybrids was established in 1953 at a different site under a bamboo lath shed. Because of the vigor of the hybrids, they were planted in unusually large beds (10 × 3 ft.) to provide more space for vine growth and root expansion. These plants commenced bearing flowers during the 1955 season, and inflorescences were recorded on the following plants: No. 9, 20, 22, 27, 30, and 33.

The reactions of 3 of the flowering hybrids in the second planting are of interest. Hybrid No. 20 blossomed consistently in the first planting until 1955, when it failed to do so. It blossomed that year, however, in the second planting. Hybrids No. 22 and No. 27 never blossomed in the first planting, though the plants reached an age of 12 years. They bore flowers in the second planting about 2½ years after the cuttings were planted.

Studies also were conducted on the flowering habits of the parents of the hybrids. In 1953, 5 large mature plants of *V. phaeantha* were observed during the flowering period. One half the flowers were pollinated, and the vines produced an average of 6.4 fruits per plant. Only 1 of these, however, was harvested as a mature fruit, since the others dropped during the period of development. Corresponding plants of *V. planifolia* in their second year of flowering had an abundance of flowers, and they retained their fruits to maturity. Similar results were obtained in 1954, when 25 mature plants of *V. phaenantha* were studied. Only 10 flowers were produced and one half of these set fruit. All of the fruits dropped before maturity. Plants of *V. planifqlia* in contrast had abundant flowers in 1954, and they retained their fruits to maturity.

From these data and general observations of previous years, it appears that plants of *V. phaeantha* flower sparsely and tend to drop their fruits before they are mature. Mature healthy plants of *V. planifolia*, in contrast can be relied on to produce an adequate supply of flowers and to retain their fruits to maturity, even under conditions that appear to be adverse for other species.

TABLE 2.—*Flowering record of 33 vanilla hybrids (Vanilla planifolia × V. phaeantha) for 4 seasons*[a]

Hybrid No.	Flowering in indicated Season[b]			
	1951	1952	1954	1955
1	—	—	—	F
3	—	F	—	F
4	—	F	—	F
8	F	F	—	F
9	F	F	—	F
15	—	—	—	F
18	—	—	—	F
20	F	F	F	—
25	—	—	—	F
30	—	—	—	F
31	—	F	—	—
32	—	F	—	—
33	—	—	—	F

[a] Plants commenced flowering in 1951, when they were 8 years old. Those plants not listed have not borne flowers to date.

[b] F = flowers, — = no flowers.

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VIRUS DISEASES OF SWEET POTATO IN EAST AFRICA. I. IDENTIFICATION OF THE VIRUSES AND THEIR INSECT VECTORS[1]

F. M. L. Sheffield

SUMMARY

At least 2 viruses attack sweet potatoes in East Africa. Virus A, found in only 1 locality, causes a relatively mild disease and is transmitted by *Myzus persicae* (Sulz.), which can acquire it in a 2-minute feeding. Virus B is widespread throughout East Africa. It is transmitted by *Bemisia tabaci* (Genn). The virus-vector relationships are obscure; under the experimental conditions used, *B. tabaci* acquired the virus in 5 minutes but it was a very inefficient vector. Virus B exists in a number of strains, the mildest of which may cause no symptoms in some sweet potato varieties whereas others cause extremely severe diseases. Infection with virus A does not protect a plant from infection with virus B, but infection with a mild strain of the latter protects against infection by a severe strain. Neither virus is transmitted mechanically to healthy sweet potatoes. Infection by virus B may be masked or not fully systemic in dull, cold conditions, but all attempts to inactivate it in the tubers before germination by heat treatment or by chilling failed. Virus A might be the same as one of the sweet potato viruses described from the United States, but virus B is distinguished from them by its insect vector.

A suspected virus disease of sweet potato (*Ipomoea batatas* (L.) Lam.) was reported from Ituri in the Belgian Congo in 1939 and then from Uganda in 1944 (4). Similar reports followed within a few years from Kenya, Tanganyika Territory, from parts of the Belgian Congo, Ruanda-Urundi, Transvaal, and Nyasaland. Nattrass (7) and Nichols (9) transmitted the causal agent by grafting and did not find any visible pathogen. White flies were suspected vectors, but Nichols failed to transmit the pathogen in a few limited experiments with these. Sheffield (14), reporting on its distribution in parts of East Africa, Belgian Congo, and Ruanda-Urundi, suggested that it had been long established in Africa. She also confirmed that the disease was due to virus infection.

For the present studies, some 26 specimens of sweet potatoes showing a wide range of symptoms were brought to the Muguga glasshouses from various parts of the 3 East African Territories of Kenya, Tanganyika Territory, and Uganda. This paper will describe some properties of the 2 viruses that have been recognized. When it became necessary to differentiate between them, they were designated A (aphid-transmitted) and B (Bemisia-transmitted) so that they could be included without confusion in any system of nomenclature which may in the future be internationally adopted.

MATERIAL.—*Healthy test plants.*—As sweet potatoes are highly heterozygous, seedlings could not be used as experimental material, and 3 apparently healthy clones of 10 varieties selected from the field and used in the early part of these studies carried latent virus. This made it necessary to develop clonal material through vegetative propagation of seedlings found to be susceptible to the virus. Sweet potato seldom sets viable seed, but 44 seedlings were produced from seed collected from 3 local varieties and from 19 varieties grown at Amani in 1944 by R. F. W. Nichols. After their suitability was tested by grafting with infected scions, 4 clones were selected that were of different female parentage and of different habit, with differently shaped leaves; however, all were free of the red pigment that may obscure symptoms and all showed obvious symptoms when infected. They were designated Muguga A, B, C, and D; their female parents were Amani 1503, Calabash leaf, Linslade, and Mundoro, respectively.

Large numbers of plants were obtained for experiment by rooting apical cuttings 2–3 in. long in sand in flats and then potting them separately in a sterilized soil mixture.

Sources of virus.—In the field, symptoms vary greatly in different varieties and may vary within 1 variety; preliminary experiments (14) showed that virus from 1 source can produce a wide range of symptoms or degrees of severity in different varieties. Difference in severity within a variety was originally thought to be due to primary and secondary infections; comparison of such specimens in the glasshouse showed this to be sometimes true but that such differences can also be due to differences in the infecting virus.

The most obvious and usual symptoms are stunting, with shortening of the internodes, reduction in leaf size, and general chlorosis. Leaves may be of leathery consistency; in the varieties with much-dissected leaves, segments may be strap-shaped. Necrotic areas and back-rolling of the leaf margins occur rarely. Yellow mottling is more obvious in the broad-leaved than in the narrow-leaved varieties; it is seldom bright in the field but may become so in the glasshouse. Bronze or purple mottling of the leaves is confined to those varieties that normally produce anthocyanin pigment strongly. As this pigment often disappears in the glasshouse, the bronze mottle does too.

Some 26 sweet potato specimens assumed to be virus infected were brought from the field. Thirteen came from a collection that had been maintained at the Kawanda Research Station, Uganda. Two were found in a native plantation near Moshi in Tanganyika Territory, whilst the remainder came from native plantations in Kenya. Seven of these were found in 3 planta-

[1] Accepted for publication May 1, 1957.

tions in the Aberdare Mountains, 1 came from the Teita Hills, another was found near Muguga, and 2 were found near the Jacaranda Coffee Research Station at Ruiru.

After a short period of propagation in the glasshouses at Muguga, some of these specimens appeared identical with others from the same area and were discarded. In a few cases, plants only mildly affected in the field were severely affected in the glasshouses. In 1 case only, a specimen taken from a severely affected plant at Kawanda showed only a mild disease in the glasshouse. Experiment showed a few of the abnormalities at first suspected of being due to virus infection to arise from other causes such as infestation with thrips or with eriophyid mites. Other abnormalities were not transmissible, and their causes could not be defined although some were suspected of being of genetic origin (14, 15). Fifteen virus-infected specimens were retained and vegetatively propagated.

It has been remarked that symptoms sometimes changed when infected plants were taken from the field to the Muguga glasshouses. At Muguga, the seasonal contrasts are exaggerated by altitude (6800 ft.); although this area is only 80 miles south of the Equator, the hot season has a mean maximum air temperature exceeding 76°F, a mean air temperature exceeding 65°F, and more than 10 hours per day of bright sunshine. The corresponding cold-season means are 64°, 56°, and 3.5 hours, respectively. It was found that plants infected in the glasshouse rapidly lost their symptoms if planted outside at Muguga in the cold season. Experiment showed that the virus may cease to be fully systemic: 9 scions taken from 1 such plant caused disease in only 2 of the plants to which they were grafted in the glasshouse. In the hot season, symptom production seems to be inhibited under glasshouse conditions: plants that had been experimentally inoculated by insects showed no symptoms until 3 weeks after they were transplanted into the field. Symptoms appeared too soon for them to have been accidentally inoculated after moving, and some plants had already been shown to be infected by grafting to healthy plants. All controls remained healthy.

TRANSMISSION METHODS.—*Mechanical.*—These methods are described briefly under the appropriate section.

Graft.—The viruses were readily transmitted by grafting. About 1–2 weeks after potting, cuttings were grafted with infected scions. Usually a top cleft graft was wrapped with wet raffia and enclosed in a polyethylene tube.

Insect.—In the search for insect vectors, field collections of insects and mites were made at approximately monthly intervals over a full year from sweet potato plots near the Jacaranda Coffee Research Station, Ruiru, Kenya. Much spread of virus disease had been observed there in 1952 (14), and this continued during the collecting period.

Preliminary trials usually were made by releasing the insects into glasshouses containing about 24 infected plants and 60 small healthy rooted cuttings. When this work was started, there was no suggestion that more than 1 virus might be concerned. In the earlier experiments, the source plants chosen were all carrying strains 1, 2, or 3 of virus B.

Insects of only 1 family were released into a house in each experiment, but invaders could not always be excluded since insecticides usually could not be used. The invaders seemed to be limited to aphids and red spiders. The experimental insects were left for several weeks; the house was then fumigated and the donor plants discarded. About 10–12 weeks from the start of the experiment, the plants were cut back. When plants are inoculated by grafting, symptoms never appear in less than 3 weeks and often do not appear for as long as 6. There was little to indicate the length of time necessary for the appearance of symptoms when the virus was transmitted by insects, except that Rosen (13) had suggested that symptoms of sweet potato mosaic disease never appeared in less than 2 growing seasons. Owing to pressure on glasshouse space, plants were sometimes put into the ground near the laboratories and remote from other sweet potatoes. Spread of virus was known to be slight at Muguga, and no plants were put out until at least 4 months from the start of the experiment. In these experiments, only white flies gave positive results and symptoms appeared whilst the test plants were still in the glasshouses.

Simultaneously with these preliminary trials, some of those insects that could be reared on sweet potato were cultured for use in more exact experiments under fully controlled conditions. Details have already been recorded briefly (16), and it is necessary here only to describe the methods used to rear those insects with which successful results were obtained.

1) *White flies.*—Cages for breeding were made on 2 × 1.5 × 2-ft. wire frames, with strips of thin metal 3 in. wide at the base and 1 in. wide at the top. The sides were covered with muslin, which was fixed to the metal strips; 1 side was provided with 2 sleeves. The top was covered with permeable cellophane. Boxes containing host plants were stood on narrow wooden strips in a water-filled deep metal tray that was a few inches larger than the cage. The cage was then put over the plants and stood in the water, care being taken that it should not touch the edges of the tray or walls of the glasshouse. White flies were picked up in a suction collector (12; Plate 68, Fig. 7) which was later opened in the cage. When required for the experiments, the insects were again removed in the suction collector. The cages were kept in a shaded glasshouse.

In most experiments, white flies that had previously fed on infected plants were transferred to small-sized rooted cuttings under lamp glasses. After about a week, and before the lamp glasses were removed, the plants were sprayed with a nicotine solution. Three to 4 weeks after the start of the experiment, any leaves bearing white fly nymphs were removed and destroyed. Thereafter, the houses were fumigated frequently. It was never found possible to eliminate all white flies,

but the second generation did not fly until about 4 weeks after the start of the experiment. In any 1 experiment, all plants which developed symptoms did so within a period of a few days and any developing symptoms 4 weeks after the majority were disregarded. Before the results were recorded, all experimental plants were tested by grafting scions to healthy plants.

2) *Aphids.*—These insects were reared on sweet potato plants under cylindrical muslin cages, with permeable cellophane tops, supported on galvanized metal frames. When aphids were fed on the test plants, the latter were covered with lamp glasses. At the close of experiments, the lamp glasses were removed and the glasshouses were immediately fumigated with benzene hexachloride.

RESULTS.—*Preliminary examination of viruses and virus strains.*—Scions from the 15 naturally infected specimens retained were grafted first to apparently healthy plants of 7 varieties obtained from the field and later to the 4 Muguga varieties. Virus from each source was thus compared on 5 plants of each of 11 varieties.

A virus from 1 source might cause a more severe disease in 1 host than in another. For example, under the conditions of the experiment, Muguga A was more seriously affected by the mild strains than were other hosts whereas Muguga B was the least seriously affected by the severe strains. When viruses from different sources were compared on the same hosts, a virus causing severe symptoms in 1 host consistently caused severe symptoms in all hosts.

The viruses fell into 6 groups that, with 1 exception, differed only in degree of severity of symptoms induced on any host. It will later be shown that 5 of these groups are strains of virus B whilst the sixth group is a different virus, designated virus A. Some typical symptoms in varieties Muguga A and C are shown in Figures 1 and 2.

1) *Virus B, strain 1.*—In all hosts, this strain caused more severe symptoms than did any other. It had also caused a very severe disease in the broad-leaved fodder variety in which it was found at Jacaranda.

2) *Virus B, strain 2.*—Virus from 6 sources caused identical diseases slightly less severe than that caused by strain B-1. The specimens had originated from 4 different districts in Kenya and Uganda, and some were from broad-leaved and others from narrow-leaved varieties that had shown markedly different symptoms.

3) *Virus B, strain 3.*—Symptoms caused by this strain were intermediate in severity between those caused by strains B-2 and B-4. It was found in only 1 sweet potato specimen, which came from Uganda.

4) *Virus B, strain 4.*—Virus from 5 sources caused the same disease, which was mild in most hosts. It originated from several sweet potato varieties, showing various symptoms, from Kenya and Uganda.

5) *Virus B, strain 5.*—This strain was found in an apparently healthy plant of the variety which yielded strain 1; it caused an extremely mild disease in other hosts. In the glasshouse, the specimen plant occasionally showed a few mild symptoms on a few leaves only. Grafting to known healthy stocks showed that these branches and only some of the apparently healthy ones contained virus. The original stock was vegetatively propagated in the glasshouses for 4 years. It then showed severe symptoms, an indication that the virus had mutated to a severe form. For this reason it was discarded.

6) *Virus A.*—In most hosts this virus appeared similar to the other forms and intermediate in severity between strains 4 and 5 of virus B. In the variety Muguga C, however, it caused large numbers of small discrete chlorotic spots on all leaves and only rarely caused any mosaic symptoms. The other forms sometimes caused a few chlorotic spots (as a first symptom) in several varieties, but these enlarged and fused to give a typical mosaic pattern. This virus was found only near Moshi in Tanganyika Territory. It was latent in a sweet potato specimen that was selected because it showed another abnormality later found not to be due to virus infection.

Microscopic examination.—Detailed anatomical and cytological examination was made of leaves, stems, and smaller roots of all the infected plants brought in from the field and also of plants of other varieties inoculated from them. No abnormalities were observed. In tubers infected with severe strains of virus B, a symptom that seems similar to that described as "corky spot" in the United States (10) was often found.

Attempted mechanical transmission to sweet potato.—The test sweet potato plants used in the following experiments were retained for 2–3 months and all remained apparently healthy.

1) *Mixed viruses.*—Composite samples, composed of leaves from each of the stock virus diseased plants, were crushed in a mortar and juice was extracted into water or into 0.5 per cent sodium sulphite. Juices were rubbed over leaves of rooted cuttings, Celite being used for half the plants. Five test plants of each of 7 local varieties were used in each of the 4 experiments.

2) *Virus B.*—The above experiments were repeated with the 4 selected Muguga varieties and juice from sweet potatoes infected with strain 2 of virus B. In

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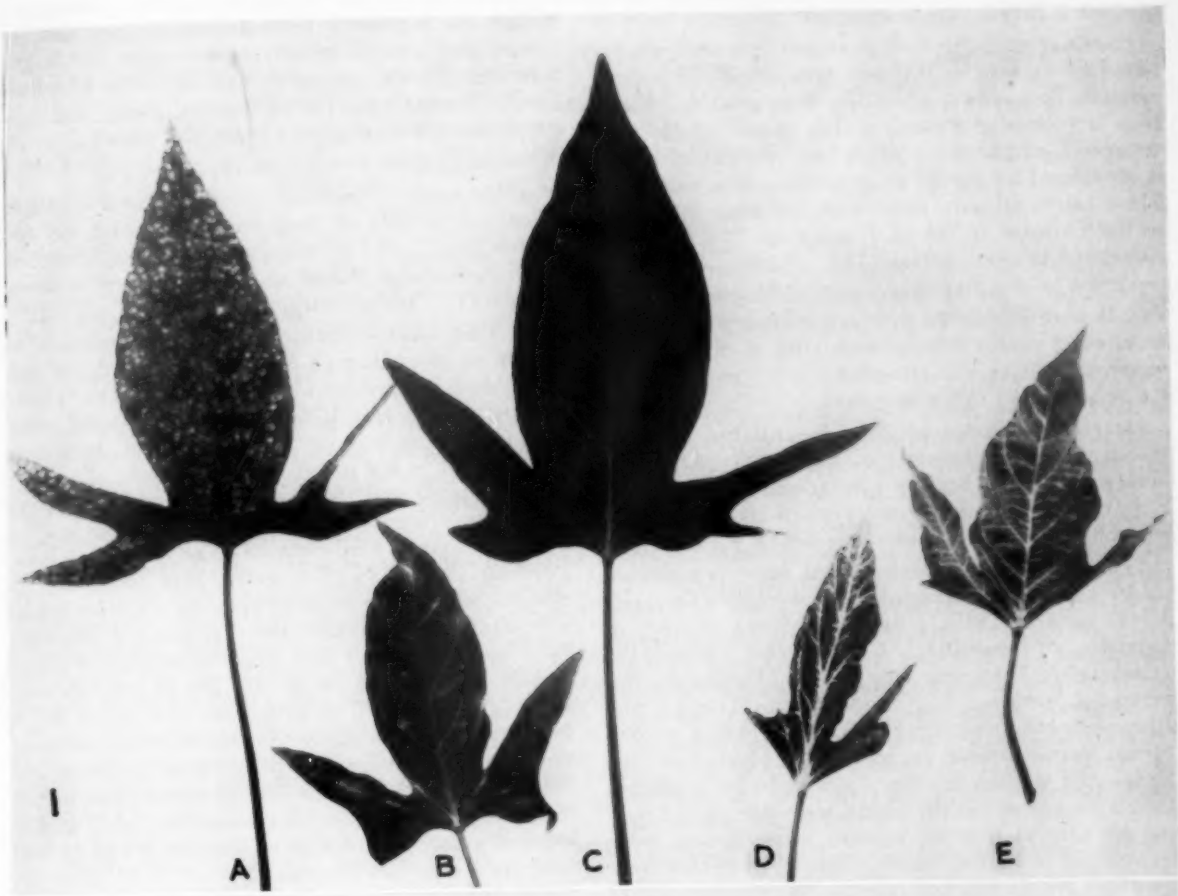
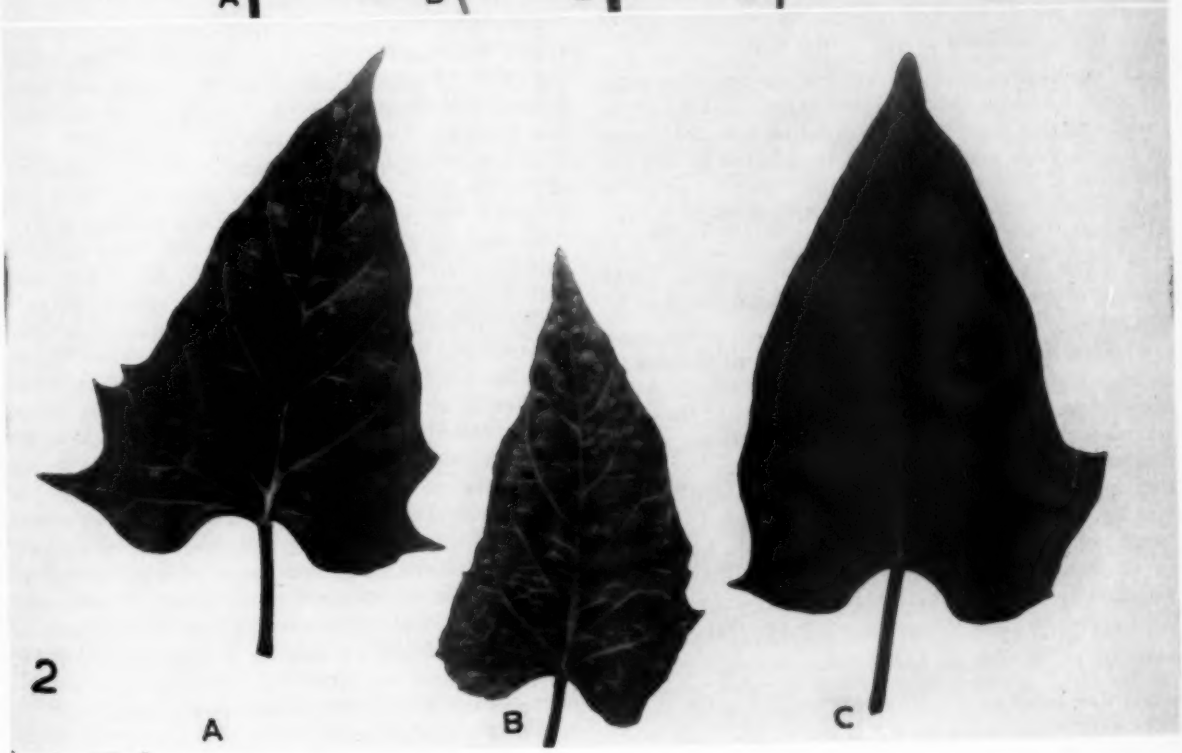
FIG. 1-2. Fig. 1. Leaves of sweet potato var. Muguga C. A) Spotting due to virus A. B) Faint mottle occasionally caused by virus A. C) Healthy control. D, E) Stunting, mottle, and distortion due to severe strains (1 and 2) of virus B. —Fig. 2. Leaves of sweet potato var. Muguga A. A, B) Infected with mild strains (4 and 3) of virus B. C) Healthy control.


1
A
B
C
D
E


2
A
B
C

a further repetition, test plants were kept in semidarkness for 5 days before inoculation.

Juice was extracted from sweet potatoes naturally infected in the field or that had been inoculated experimentally by means of white flies or by grafting. Juices from a species of *Petunia* and a species of *Ipomoea* (near to *I. purpurea* L.) which had been experimentally inoculated by insects or by grafting also were used. These juices all gave positive results when inoculated to the *Ipomoea* sp. or to *Petunia* sp. but not when inoculated to sweet potato (17).

3) *Virus A.*—Five plants each of Muguga A, B, C, and D were inoculated with sap extracted into water from sweet potato infected with virus A. In a second experiment, juice was extracted into 0.5 per cent sodium sulphite and Celite was used.

Insect transmission of virus B.—All the following insects, except *Trionymus* sp., were set free in glasshouses containing healthy plants and plants infected with virus B. In the cases specified, more carefully controlled experiments were also done.

1) *Aphids.*[2]—The species tested were *Aulocorthum vincae* (Walker), *Macrosiphum euphorbiae* (Thomas), *Myzus persicae* (Sulz.), *Aphis craccivora* (Koch), *A. solanella* (Theobald), *Chaetosiphon fragaefolia* (Cockerell), *Dactynotus compositae* (Theobald), *Hypermyzus lactucae* (L.), *Myzus ornatus* (Laing), and *Rhopalosiphum maidis* (Fitch). The first 3 species named were reared on caged infected plants and then transferred to small healthy plants under lamp glasses. Others, reared on healthy plants, were starved and then fed for a short time on infected leaves before being transferred to healthy plants. Ten to 20 aphids were used in each of 140 experiments. No transmission of virus B was obtained in any of the tests.

2) *Leafhoppers.*—Six unidentified species were used; all were found on sweet potato but only 1 bred on it. Insects of this species were reared on infected shoots in muslin bags and were then transferred to healthy shoots in similar bags. Leafhoppers were used in 30 transmission tests, each of which involved 10–20 insects. All results were negative.

3) *Frog hoppers.*—One species occasionally found feeding on sweet potato but which did not breed on it, was tested without success.

4) *Mealybugs.*—Two species, both of which bred on sweet potato, were used. One was identified by A. R. Melville as *Planococcus Kenyae* (Le Pelley); the other was thought by G. de Lotto to be *Trionymus* sp. *P. Kenyae* was reared on infected plants and insects were transferred, 10 at a time, to 92 healthy plants. *Trionymus* sp. was found only on the underground parts of sweet potato and was therefore unsuitable for testing by the cruder method. It was reared in the dark on sprouted Irish potatoes. Small sweet potato cuttings were rooted in sand through glass tubes that were 1.5 in. long and 0.5 in. in diameter. When roots had formed, the bottom of the tube was plugged and the cutting was planted in soil. Insects were put in the tube, the top was plugged, and the tube was then buried. Insects were fed on infected plants and then transferred 10–20 at a time to healthy plants.

Neither species transmitted the virus.

5) *Mites.*—Two species of red spider and 2 eriophyid mites were tested; all bred on sweet potato. Of the 2 latter, one caused bronzing and the other, identified by G. O. Evans as *Aceria* sp., caused erinose of sweet potato (15). Neither transmitted the virus.

6) *White flies.*—Fifteen small, rooted cuttings of each of varieties Muguga A, B, C, and D were put into a glasshouse together with 25 sweet potato plants infected with strains 1, 2, or 3 of virus B, and white flies found breeding on sweet potato at Jacaranda were set free in the house. Six weeks later, the house was fumigated and the donor plants and any leaves of the experimental plants observed to be bearing white fly nymphs were removed. The house was fumigated at intervals and a watch kept for white fly nymphs, which were removed immediately when found. The plants were cut back twice at monthly intervals. Four months after the start of the experiment, 2 plants showed symptoms of the disease and in the following month, 13 others. The plants were retained for a further month in the glasshouse, but no more developed symptoms. All were tested by grafting scions to healthy rooted cuttings, and only scions from the 15 with symptoms induced disease in the stocks. The infected plants were 3 each of Muguga B and D, 4 of Muguga A, and 5 of Muguga C.

This virus has now been transmitted by white flies from 2 different localities, Jacaranda and Kinoo, which are about 25 miles apart. White flies from both these sources were sent to Dr. L. Russell of Washington, who identified them as *Bemisia tabaci* (Genn).

In a further preliminary experiment, the possibility of the presence of insects other than white flies was excluded. An infected plant from the previous experiment was put into a white fly breeding cage together with 25 small healthy rooted cuttings. White flies from both sources, about 500 in all, were added. After 4 weeks, the plants were removed, sprayed with nicotine, repotted, and placed in an adjoining glasshouse. Plants were carefully watched, and any leaves bearing white fly nymphs were removed. After 4 months, none of the plants showed symptoms and all were cut back. After a further month, symptoms developed (within a period of 15 days) in 16 of the 25 experimental plants. The plants were kept for another month, but no others developed symptoms. Virus was recovered, by grafting to healthy stocks, only from plants showing symptoms.

Subsequent experiments were more detailed and exactly controlled. Owing to the long period elapsing between inoculation by white fly and the appearance of symptoms, these experiments were difficult to plan and often would have been differently designed had the results of those immediately foregoing been available.

[2] The last 7 species were not found on sweet potato nor would they breed on it. All aphids were identified by Dr. V. F. Eastop.

TABLE 1.—*Transmission of virus B by different numbers of white flies from 2 different sources*

No. of white flies	Origin of white flies [a]	Duration of acquisition feeding	Duration of infection feeding	No. of plants infected [b]	
				Experimental	Control
10	K	Indefinite [c]	14 Days	0/7	0/4
10	J	4–5 Days	4–5 Days	3/13	0/6
20	J	4–5 Days	7 Days	2/11	0/5
20	K	4 Days–indefinite	7–18 Days	4/26	0/8
50	J	6 Days	7 Days	7/18	0/8
100	J	Indefinite	7–18 Days	29/43	1/20
100	K	Indefinite	7–18 Days	14/25	0/13
100	J, K	Indefinite	6–10 Days	10/34	0/15

[a] K = Kinoo, J = Jacaranda.
[b] Numerator shows number infected; denominator, total number of experimental plants.
[c] Reared on infected plant.

Effect on transmission of virus B of number and source of white flies.—Table 1 was compiled from the accumulated results of a number of experiments not done simultaneously. In all, the white flies had a long feeding period on the infected plant or were cultured on it. The donor plants were ones inoculated by white flies in previous experiments. Table 1 shows that white flies obtained from the 2 localities were about equally effective in transmitting the virus (Table 1) and that a large number of insects were necessary if a high proportion of infections was to be obtained.

Transmission of virus B after short acquisition feeding periods.—A muslin tube, held open at the middle by a wire ring, was tied with tape at both ends and attached at the top to a stake placed beside a young healthy rooted cutting. A known number of white flies was inserted through the top of the tube. They were left to starve for a known period, and then a small infected shoot was inserted into the tube. Subsequently, the insects were shaken off the shoot, which was then removed. The base of the tube was immediately tied over the young healthy cutting. After a week, the white flies were killed and the tube removed. In the first experiment, in which 10 plants were used, 100 white flies were put into each tube after they had been starved for periods varying from 3½ to 6 hours. They were allowed to feed on the infected shoot for either 5 or 10 minutes. Five of these plants became infected, as did 1 of 5 controls. In a second similar experiment, only 20 white flies were used on each plant and none of 10 test plants or 5 controls became infected.

This method was adopted because it is almost impossible to watch a white fly feed and to time its feeding. If in contact with an infected shoot for 5 minutes, few were likely to feed for that length of time; some might feed for lesser periods and some not at all. The results show that white flies can acquire virus B in 5–10 minutes, but the probability of their doing so is very low. No further work was done on these lines as the technique was elaborate and appeared from the practical standpoint to have no advantage over rearing the vectors on infected plants.

Time elapsing between infection with virus B and appearance of symptoms.—Although in all the insect transmission experiments test plants were cut back in an attempt to force the appearance of symptoms, these never appeared in less than 3 months and often not until 6 months had elapsed.

The period is probably similar under field conditions. At Jacaranda in 1954 at the beginning of January, no white flies were found; during the dry hot weather of February and March, however, they were present in such great numbers that they rose in a cloud when the plants were disturbed. With the start of the rains, the numbers decreased until none at all could be found in June. The population again increased in August and September but did not seem to reach the same peak as that observed earlier in the year. In November, the population again fell, and none at all was found in December.

In 1 small plot, all plants appeared healthy until the middle of May when 1 infected plant was observed. A month later, a small group of infected plants surrounded the one earlier seen to be infected. The number of obviously infected plants then increased each month until November, when about half showed symptoms. After this, no further increase was apparent.

The first infection thus became obvious about 4 months after the appearance of white flies, and the number of infected plants did not increase 5 months after the complete disappearance of white flies in June. If the vines were not infected when planted, the time elapsing between inoculation by insects and the appearance of symptoms seems to be about 3–6 months in the field as it was in the glasshouse.

White fly transmission of virus B from different sources.—Some small experiments were done to find out whether apparently similar viruses obtained from different sources were all transmitted by the same vector. The original stock plants brought from the field had been continuously propagated vegetatively, and white flies were reared on rooted cuttings from these. They were then transferred to healthy cuttings where they were left for 7 days. When these experiments were begun, the necessity of using large numbers of white flies was not realized so only 10 or 20 were put on each plant. The number was later increased to 50 and then to 100.

TABLE 2.—*White fly transmission of virus from different sources*

Virus and strain	Source	No. of plants infected[a] following inoculation by means of indicated no. of white flies[b]			
		10	20	50	100
A	Moshi				0/10
B1	Jacaranda	0/5		0/2	3/5
B2	Kawanda	0/10	1/10		
	Kawanda[c]			0/2	2/5
	Aberdare Mts.	2/15	1/10	0/2	
	Muguga	1/5		0/2	4/5
B3	Kawanda	0/10	2/10	0/2	2/5
B4	Kawanda				2/5
	Kawanda[c]			0/2	1/5
	Aberdare Mts.				1/5
B5	Jacaranda				2/5

[a] Numerator is number of plants infected and denominator is number inoculated. Number of control plants was approximately half that of inoculated plants; none became infected.

[b] Insects were reared on infected plants and were allowed to feed for 7 days on the test plants.

[c] Virus from a different sweet potato variety from that immediately above.

With the exception of virus A, all the forms tested were transmitted by white flies (Table 2). Similar results, were obtained when another species of *Ipomoea* was used as the recipient host plant (17).

Transmission of virus A by aphids.—Experiments were made to find out if virus A and also 2 other mild virus forms, which had not then been transmitted by white flies, might be carried by aphids (*Myzus persicae*).

In the first experiments, the feeding times used were those employed by Webb and Larson (21) in the experimental transmission of feathery mottle virus (i.e. 3 days on both donor and healthy plants) but were later varied as is shown in Table 3. Of the virus forms tested, only virus A was carried by *Myzus persicae*. Symptoms appeared in 3–4 weeks after inoculation, and no control plants became infected.

The fact that good transmission was obtained in 1 experiment in which starved insects were allowed an acquisition period of 2 minutes suggests that the virus belongs to the nonpersistent group.

Protection of a plant by a mild virus strain from infection by a severe virus strain.—Before the insect vectors were known, some attempt was made to carry out cross-protection experiments by grafting. Ten small-sized rooted cuttings were inoculated with strains 4 or 5 of virus B and 10 with virus A, and all became infected. All were later inoculated with strains 1 or 2 of virus B. Healthy controls and controls infected with each virus form separately were kept. When either strain 1 or strain 2 was added to strains 4 or 5, symptoms remained mild in about half the plants; when either strain was added to virus A, all plants developed more severe diseases than did the controls inoculated with the severe strain only.

Some of the milder forms were then added together in pairs, in tests in which the 4 Muguga varieties were used as stock plants and the viruses were strains 3, 4, or 5 of virus B and virus A. Scions infected with the various virus forms were grafted simultaneously in pairs to 3 plants of each variety, all possible combinations being used. Three plants of each variety were also inoculated with each virus strain separately. Whenever virus A was included in a pair, the resultant disease was more severe than that induced by either component alone. With the other pairs, the disease varied but was never more severe than that caused by the more severe component.

Further cross-protection experiments were made later, the severe virus form being introduced by the insect vector.

White flies were cultured on a plant infected with virus B, strain 1; 100 were transferred to each of 50 small healthy rooted cuttings of Muguga C and to each of 50 similar cuttings from a plant previously inoculated by grafting with virus A. After 7 days the white flies were killed. Ten healthy controls and 10 infected with virus A were kept. After 3 months, symptoms appeared on a few of the originally healthy plants, and some of those originally infected with virus A were now more stunted than were those that were healthy when inoculated. They showed both the spotting

TABLE 3.—*Transmission of virus A by Myzus persicae*

Virus and strain	Period of starvation of insects (hours)	Duration of acquisition feeding	Duration of infection feeding (days)	No. of insects per plant	No. of plants infected[a]
A		3 Days	3	40	4/10
A		3 Days	3	30	2/10
A	20-21	2 Minutes	10	10	5/5
A	19-23	2 Minutes	8	10	5/8
B5		3 Days	3	40	0/10
B4		Indefinite[b]	8	20	0/10
B5		Indefinite	8	20	0/10
B4	19-24	2 Minutes	6	10	0/5
B4	20-23	2 Minutes	9	10	0/10
B5	18-20	2 Minutes	9	10	0/5
B5	20-23	2 Minutes	8	10	0/10

[a] Numerator shows number infected; denominator, total number of experimental plants. Controls numbered half the experimental plants; none became infected.

[b] Reared on infected plant.

characteristic of virus A in this variety and also a severe mottling and distortion. After 4 months, when the plants were discarded, the position was as follows: of 50 plants originally healthy, 10 showed symptoms characteristic of infection by strain 2 of virus B and 40 were apparently healthy; of 50 plants originally infected with virus A, 12 showed the severe symptoms mentioned above and 38 those of virus A only; all controls remained unchanged.

These results were checked by grafting scions from the experimental plants to healthy cuttings.

A further experiment was made similar to the above, except that only half the numbers of plants was used and virus A was replaced by strain 4 virus B. After 4 months, 7 of the plants that were healthy when inoculated with the second virus had severe symptoms, whereas plants infected with the mild strain before inoculation with the severe strain remained indistinguishable from the infected controls. One healthy control developed severe symptoms.

Although the numbers of plants successfully inoculated in both these experiments were small, the results suggest that strain 4 will protect against infection with strain 2 of virus B but that virus A is unable to do so. In fact, viruses A and B appeared to be synergistic.

Attempted therapy.—Before attempts could be made to inactivate the severer strains of virus B by heat treatment, it was necessary to determine the method least deleterious to the tubers. When 10 tubers from healthy plants were used in each experiment, all germinated after being held at 52°C for 20 minutes, either in water or in a moist atmosphere. None survived holding at 52° for 2 hours in a moist atmosphere, but 8 survived when the treatment was in water. All subsequent treatments were given in a water bath.

Batches, each of 10 infected tubers, were treated for 1, 2, or 3 hours at 45°, 48°, 50° or 52°C and for 4 hours at 45° or 48°C. None survived the longest period at any temperature, and none survived being held at 54° for 30 minutes.

In each of 4 further repetitions, 10 tubers were treated for 2 hours at 52°C, and the numbers surviving in the 4 tests were 5, 3, 5, and 6. When 20 tubers were treated for 2½ hours at 52°C, only 2 survived. The sprouts from all the tubers that germinated after any of these treatments showed symptoms of virus infection. Five controls were used for each experiment, and all developed symptoms when sprouted.

As infected plants became symptomless or the virus was not fully systemic in cold climates, it seemed possible that chilling the tubers might inactivate the virus. For each experiment, 10 tubers were kept in a refrigerator at 4°C before being germinated in sand. In the 11 experiments done, the time of chilling varied from 1 to 20 days. None of the tubers survived chilling for 20 days, and the sprouts from 3 that germinated after 18 days' chilling showed symptoms as did all those that germinated after shorter treatments.

Discussion.—The results show that sweet potatoes in East Africa are infected by at least 2 viruses. One, virus A, is carried by *Myzus persicae*, and only 1 strain of it is known, whereas virus B, carried by *Bemisia tabaci*, exists in a number of strains.

Virus diseases of sweet potato have been described from several other parts of the world, but it is not clear whether any are identical with those found in East Africa. Symptoms are obviously not a reliable basis for comparison, as they differ in different varieties of the host and may vary with the environment or with the virus strain. Nothing is known of the chemical or physical properties of any of the viruses, so the only criterion is the little information available on insect vectors and their interrelationships with viruses and hosts.

The first record of a virus disease in this host seems to be that of Ensign (3), who described a mosaic that stunted the plants and reduced yielding ability. Recent records from the United States refer to 2 much milder diseases causing little or no loss of yield. Aphids are vectors of both feathery mottle virus (21) and corky spot virus (5). Wellman (22) found cucumber mosaic virus occurring naturally in sweet potatoes in the field and also transmitted it to them experimentally by means of aphids, but he was unable to transmit it mechanically to sweet potato. Virus A can be distinguished from feathery mottle virus in that the latter is easily transmitted mechanically. It is similar to cucumber mosaic virus in that neither has been transmitted mechanically to this host and both are acquired by their aphid vectors in very short feeding periods (20).

Virus B is distinguished from all these viruses by its insect vector, although it does cause a condition in the tubers similar to that resulting from infection with corky spot virus. "Ishuku-byo" (19) and mosaic mottle (8) are severe diseases occurring in the Ryukyu Islands and in Ceylon, respectively. Adsuar (1) has described a disease in Puerto Rico as being similar to the sweet potato virus disease reported from East Africa. These comparisons are based only on the severity of the diseases caused, and it is not possible to identify virus B with certainty with any other attacking sweet potato.

Spread of sweet potato virus B is rapid in the field at certain altitudes. It cannot, however, be assumed that white flies are efficient vectors, for at certain seasons they are present in very great numbers. The apparent absence of spread at low altitudes can no longer be attributed to absence of the vector (14), but there is some evidence to suggest that it may be due to masking of the symptoms in the plant. It might be caused by inactivation of the virus in the insect. At high altitudes, apparent spread of virus is slight. This probably is because white flies are relatively few at such altitudes. Also, the virus is known to cease to be fully systemic in the plant when it is grown under dull, cold conditions.

Under experimental conditions, the vector is very inefficient. Other viruses, such as those of leaf curl of cotton (6), Abutilon mosaic (11), and mosaic of

Euphorbia prunifolia (2), can be transmitted experimentally by a single white fly, and the use of 10 on each plant results in all plants being infected. If 10 insects are used to transmit sweet potato virus B, the number of infections resulting is negligible and, even when 100 are put on to each plant, many still escape infection. Storey and Nichols (18) found that to transmit the virus of mosaic disease to cassava, the white fly vectors must feed on leaves less than one quarter grown. In my experiments, the insects had the opportunity of feeding on any part of a small cutting consisting entirely of immature tissue.

As attempts to inactivate the virus in tubers by temperature changes were not successful, other means of control must be sought. If a virus is not mechanically transmitted, to prevent its spread in a susceptible variety it is necessary to remove either the source of inoculum or the insect vector. It is unlikely that any economic method of control of white fly exists. Where the crop is propagated from tubers, it should be possible to reduce the incidence of the disease if those that produce visibly infected sprouts are carefully rogued. In East Africa, where sweet potato is essentially a native crop, it is propagated from long vines. Obviously infected material would be rejected as being too short; however, as symptoms are slow in appearing, many of the vines planted at the beginning of the rainy season might have become infected during the previous months when the vectors were plentiful and might not show the effects for a considerable time after planting. During this period, they would be a potential danger to neighboring healthy plants. The only method of control seems to lie in the finding or breeding of resistant varieties. The Uganda Department of Agriculture has already found 1 variety, Namujuna, that is moderately resistant to virus B and that is now grown extensively in certain districts.

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INFLUENCE OF ENVIRONMENTAL FACTORS ON THE DEVELOPMENT OF WHEAT BUNT IN THE PACIFIC NORTHWEST. I. EFFECT OF SOIL MOISTURE AND SOIL TEMPERATURE ON SPORE GERMINATION [1]

Laurence H. Purdy and E. L. Kendrick[2]

SUMMARY

Spore germination tests at various soil moisture levels were run at 5 temperatures ranging from 5° to 25°C. at 5° intervals. After 4 days' incubation, spore germination had occurred only at 15° and 20° and at the higher soil moistures. After 5 days, the best germination at all soil moisture levels occurred at 15°. At the end of 10 days' incubation, germination was equally good at 5°, 10°, and 15° at the higher moisture levels; at 20° and 25°, germination was relatively low at all moisture levels. Available moisture may be more of a limiting factor to spore germination at high than at low temperature.

In the Pacific Northwest, *Tilletia caries* (DC.) Tul. and *T. foetida* (Wallr.) Liro, the causal agents of common bunt of winter wheat, may be transmitted from 1 crop to the next either by seed-borne or soil-borne spores. Seed treatment controls bunt that originates from seed-borne spores but is only partially effective against infection by soil-borne spores. Consequently, wheat bunt in this region is perpetuated primarily by soil-borne spores, which are believed to retain their infective capacity for 1 season only. Thus, annual reinfestation of the soil is prerequisite to a recurring incidence of bunt, and this usually is assured where spore clouds from harvest operations deposit spores of the smut fungi on the summerfallow ground where the next crop is to be grown.

The degree of reinfestation, which is directly related to the level of infection in the preceding crop, usually determines the general level of infection in the next crop. Thus, a year of high bunt incidence or one of low incidence will usually be followed by another of its kind, unless, as sometimes happens, factors influencing spore germination and/or infection intervene to interrupt this pattern of bunt development. This happened in a period of high bunt incidence extending from 1950 through 1956. With 28 per cent of the harvested grain from the 1952 crop grading smutty, this presumably had set the stage for another year of high incidence in 1953. Instead, the bunt incidence in 1953 was only 4 per cent. Since such a limited amount of inoculum was produced, there should have been an even lower incidence in 1954; however, the incidence of bunt in the 1954 crop returned to a relatively high level as shown by the fact that 22 per cent of the harvested grain graded smutty.

Although no satisfactory explanation has been offered for these deviations from the usual pattern of smut incidence in the Pacific Northwest, some speculation has centered around the possible effects of an adverse moisture and/or temperature relationship during the 1952-1953 crop season. In this connection, it may be significant that within this region certain localized areas are recognized as chronic smut centers whereas adjacent areas remain relatively smut free. For example, the Palouse area of southeastern Washington, with upwards of 20 in. of annual precipitation, consistently has a high incidence of smut whereas usually only a trace of smut develops in an adjacent area in central Washington that has less than 10 in. of annual rainfall. Thus, there is strong circumstantial evidence that moisture exerts a predominant influence on the development of bunt in this region, although experimental evidence emphasizes the influence of temperature.

It is also notable that all environmental studies with respect to bunt thus far have dealt with infection of the host and the further development of the pathogen therein. No attention has been given to the effect of soil temperature and soil moisture on spore germination, particularly with respect to specific pathogenic races of the pathogens. Since spore germination in the soil or on the seed is prerequisite to infection, the factors affecting their germination are of prime importance in the life cycle of the bunt fungi. Hence, the initial step of this study was directed towards determining the influence of various moisture and temperature combinations in the soil on the germination of the spores of 1 race of *T. caries*.

LITERATURE REVIEW.—An extensive review of the literature dealing with factors influencing infection by the bunt fungi and their development in the host was published in 1941 by Holton and Heald (5). From this review it is evident that infection and the development of bunt are favored by the following factors: 1) temperatures ranging from 5° to 15°C, with lower and higher temperatures being detrimental to infection; 2) soil moisture ranging from 15 to 60 per cent of water-holding capacity, with the optimum soil moisture varying according to the soil type (the relationship to permanent wilting point, moisture equivalent, or some other reference point was not indicated); 3)

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sandy soils and humus-rich soils (as compared to clay, peat, and highly acid soils, which are detrimental to infection).

According to Mackie (10), spores of the bunt fungi require a higher percentage of soil moisture for germination than do wheat seeds, which suggests that a soil can be moist enough to sprout the wheat but too dry to germinate bunt spores; however, these statements were not substantiated by experimental data. Hungerford (6) demonstrated experimentally that low soil temperatures (9°–12°C) and fairly high soil moistures (22 per cent moisture, with a moisture equivalent of 20.7 per cent) favored high incidence of bunt. He was unsuccessful in attempts to determine the effect of soil moisture on spore germination in field experiments.

Lowther (9) found that moisture content of agar media influenced the rate of spore germination. He controlled the moisture content by varying the percentage of agar in the medium. At 16°C, germination was best on a 2.5 per cent agar medium and decreased in rate as the agar content was increased.

In temperature and soil humidity studies in Hungary, Bohus and Podhradszky (2) reported a definite connection between the distribution of the 2 bunt fungi *T. foetida* and *T. triticoides* (*T. caries*) and regional rainfall. Although the optimum temperature for germination of the spores of both species was 16°–18°C, *T. triticoides* required more moisture for optimum germination than did *T. foetida*.

Much of the early work on the relationship between soil moisture and disease development has failed to take into account the fact that soil moisture cannot be maintained at values less than field capacity in a soil mass supporting growing plants (7). Several workers (1, 4, 8, 14) have attempted to control soil moisture at points less than field capacity (7) by weighing the can plus soil and plant, then adding water to the soil surface in order to bring the system to a predetermined weight corresponding to a particular moisture content. This method wets only a portion of the soil near the surface to a field capacity, whereas the lower portion of the soil remains in the same moisture state as before the addition of water (7). Furthermore, the terms used in reporting these moisture conditions have been vague and in many cases the methods used for their determination have not been given.

Terms such as water-holding capacity, saturation point, maximum water-holding capacity, etc., have been used in phytopathological literature, and moisture contents have been reported as percentages of these figures. The exact meanings of these terms are generally obscure, and soil-moisture contents based on them often cannot be related to the moisture equivalent, 1/3-atmosphere value or permanent wilting point (7), and "available water," all of which are terms recognized and used by research workers in plant and soil-moisture relationships.

The "moisture equivalent" (7) was used by Hungerford (6) as a basis with which to compare the moisture contents of field or greenhouse soils. This constant is approximately equivalent to the field capacity of many soils. The 1/3-atmosphere value (12) is the percentage of water, on a dry-weight basis, held by a soil against a pressure of 1/3-atmosphere and is also similar to field capacity. The 15-atmosphere value (11) is the percentage of water, on a dry-weight basis, held by a soil against a pressure of 15 atmospheres and is approximately equivalent to the permanent wilting point. Moisture contents, on a dry-weight basis, when compared with the permanent wilting point and either the moisture equivalent or 1/3-atmosphere value for a particular soil, indicate the apparent availability of the moisture for plant growth.

MATERIALS AND METHODS.—In this study, a Palouse silt loam soil was used that had a 1/3-atmosphere value of 24.0 per cent and a 15-atmosphere value of 10.8 per cent. These constants, determined in the soil physics laboratory of the State College of Washington[3], were based on methods described by Richards and Weaver (11, 12).

Since the 1/3- and 15-atmosphere values correspond approximately to the field capacity and permanent wilting point, respectively, the range between these values represents the moisture available to a growing plant, or the "available moisture" (7). Moisture levels of 9, 11, 13, 18, and 24 per cent, on a dry-weight basis, were used. These moisture contents were chosen so that they would be below (9 per cent), the same as (11 per cent), and slightly above (13 per cent) the 15-atmosphere value. The next level, 18 per cent, was approximately the midpoint of the available moisture range, and the 24 per cent level represented the top of this range or approximately field capacity.

A large sample of soil was screened and then air dried; its air-dry moisture content then was determined. Samples of soil were brought up to a desired uniform moisture content by atomizing the required volume of water onto the soil sample in a rotating drum (3). The moisture content of these soil samples varied from the calculated value by no more than 0.4 per cent. Weighed samples (300 g) of the moistened soil were immediately placed in pint friction-top cans in which spore germination tests were made. This method of wetting the soil provided uniform distribution of the moisture and overcame the difficulty of trying to wet the soil to less than field capacity by adding water to the soil surface.

A water suspension of spores of Race T-5 of *T. caries* (13), to which a small amount of wetting agent had been added, was placed on glass microscope slides and allowed to dry. Two slides were then placed in each can of soil by easing them into the soil at an angle; pressure was kept on the bottom side of the slide to minimize the loss of spores from the top surface. When approximately 2/3 of the slide was below the soil surface, soil was packed against the

[3] Grateful acknowledgment is made to Jack Hsieh for determinations of the 1/3- and 15-atmosphere values.

slide sufficiently to insure good contact. The tops of the cans were replaced so as to prevent loss of moisture by evaporation.

Spore germination tests at each moisture level were run in triplicate at 5 temperatures ranging from 5° to 25°, at 5° intervals. The tests were repeated 3 times. Observations for spore germination were made at the end of 4, 5, and 10 days. The criterion for spore germination was the presence of whorls of primary sporidia on the slides. Because of the difficulty of distinguishing individual spores, germination percentages were based on the average of separate estimates by each author.

Results.—The results from the 3 experiments were generally in agreement (Fig. 1). These results show a relationship between soil moisture level, soil temperature, and rate of spore germination.

Good germination occurred at 15° and 20° in 4 days at the 18 and 24 per cent moisture levels. In 5 days, germination at 10° was nearly as high as at 15° at all moisture levels and was greater than that at 20°. At 20°, germination percentage increased only slightly after the fourth day. Germination had just begun after 5 days at the 2 temperature extremes of 5° and 25°, and this was only at the 2 higher moisture levels.

The interaction of temperature and soil moisture was most striking at the end of 10 days' incubation. In this length of time, germination was equally good at 5°, 10°, and 15° at moisture levels of 13 per cent and above; the germination averaged well above 50 percent. At 20° and 25°, germination was considerably lower than that at the lower temperatures. Although there was a progressive increase in germination percentage with each increase in moisture content at nearly all temperatures, available moisture appeared to be more critical for spore germination at high temperature than at low temperature. At the 11 per cent moisture level, germination was good at 5° and 10°, relatively poor at 15°, and only sparse at 20° and 25°. A similar trend was apparent at all moisture levels above 11 per cent, becoming progressively less striking with each increase in moisture content. At the moisture level of 11 per cent, the available moisture apparently was not adequate to support good germination at 15° and above.

These data show further that spore germination was inhibited at the higher soil temperatures (20° and 25°). At these temperatures, about one half as many spores germinated in 10 days as germinated at 5–15° at the 2 higher moisture levels. Even at the optimum temperatures for spore germination (10°–15°), available moisture at the 9 per cent level was insufficient to support germination. In contrast, good germination occurred in 5 days at these same temperatures (10°–15°) at moisture levels above 11 per cent. Hence, abundant infective inoculum would be available in a short time under these conditions; however, the possibility exists that wheat seed in soil of high moisture content (near the field capacity) and high temperature (20°–25°) may germinate and produce plants so rapidly that they escape infection.

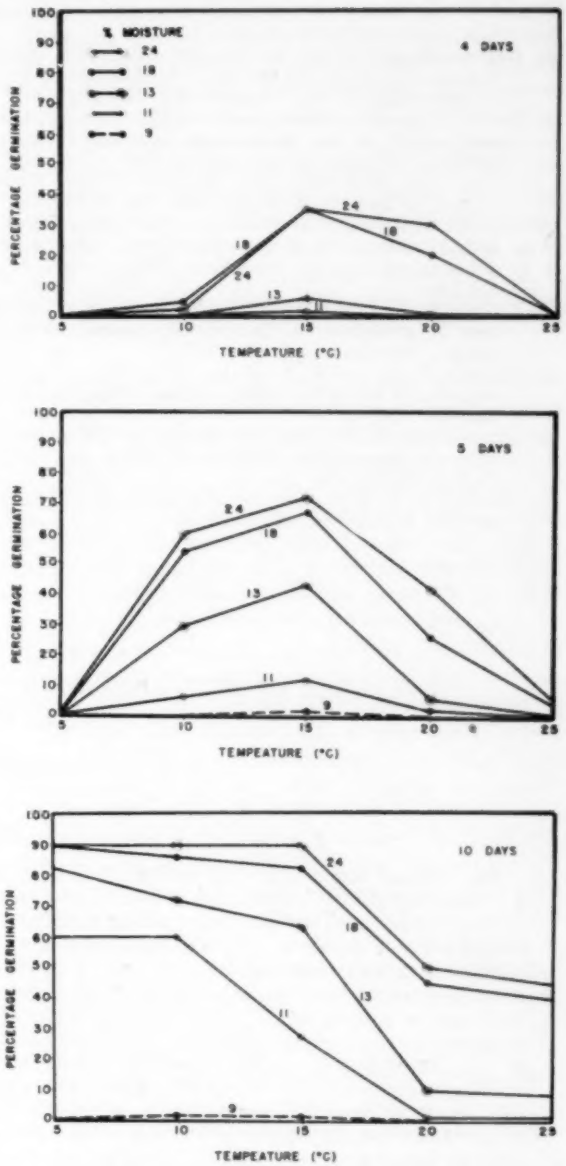

Fig. 1. Percentage germination of spores of *Tilletia caries* in a Palouse silt loam soil of 5 different moisture contents (9, 11, 13, 18, and 24 per cent) and 5 different temperatures (5°, 10°, 15°, 20°, and 25° C) after 4, 5, and 10 days.

An important factor in the development of bunt under field conditions is that wheat seedlings in infested soil may escape infection if the soil moisture and/or the soil temperature were unfavorable for spore germination. There remains for consideration also the precise relationship between these environmental factors and the infection process; this is now under investigation.

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INFLUENCE OF SOIL AND AIR TEMPERATURE ON VERTICILLIUM WILT OF TOMATO [1]

L. V. Edgington and J. C. Walker

SUMMARY

The optimal temperature for growth in culture of 3 "dauermycelial" isolates of *Verticillium albo-atrum* was 22° C. They grew very slowly at 28°, and no growth was visible at 32°. The optimal temperature for 3 pseudosclerotial isolates was 24°. They were slightly retarded at 28°, and 2 of the isolates made slight growth at 32°. All 3 "dauermycelial" isolates incited marked symptoms on tomato plants at soil temperatures of 20° or 24° combined with air temperatures of 16°, 20°, or 24°; symptoms were less severe at these soil temperatures when the air temperature was 28° than when it was lower. When the soil temperature was 28°, the disease was slight at air temperatures of 16°, 20°, and 24°. When soil and air were both at 28°, no symptoms appeared. By contrast, the 1 pseudosclerotial isolate that was pathogenic on tomato induced marked symptoms at all combinations of soil and air temperatures used. The 2 pseudosclerotial isolates from cotton induced severe disease on cotton plants when air and soil temperature were both at 28°.

When the influence of soil temperature and air temperature on disease development in tomato plants was evaluated, each factor had a significant effect, but soil temperature was the more influential. When soil temperature was lower than air temperature, the temperature within stems of tomato plants growing in the greenhouse was influenced predominantly by soil temperature during the day and by air temperature during the night. As light intensity increased, the rate of transpiration also increased; concomitantly, the effect of soil temperature on stem temperature also increased. The influence of low soil temperatures on internal stem temperature was correlated with the rate of movement of water from the soil into the roots and upward through the stem.

Results of investigations of the temperature range over which Verticillium wilt of tomato develops have varied depending on the isolate and the method of temperature control. Among the pathogenic species of *Verticillium* there are 2 important types (based on cultural characters): 1) the type that produces pseudosclerotia, which are small black sclerotium-like structures arising from monohyphal budding; and 2) the type that produces dark resting mycelium, often referred to as "dauermycelium." Some authors, e.g. Isaac (4), refer the former type to *Verticillium dahliae* Kleb. and the latter type to *V. albo-atrum* Reinke & Berth., whereas some others, e.g. Presley (7) and Rudolph (8), consider that both belong to the latter species. An optimal temperature of 22.5° C and a maximal temperature of 30° have been reported for the

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"dauermycelial" type in artificial culture (1, 4, 6), whereas an optimum of 22.5–25° and a maximum of 32–35° (4, 6) have been reported for the pseudosclerotial type. Two "dauermycelial" isolates that produced a "few" pseudosclerotia were reported to have temperature ranges similar to that reported for the pseudosclerotial type (2, 6).

Temperature studies on Verticillium wilt in the greenhouse have been of 2 types: 1) those in which plants were grown in houses having different mean air temperatures and 2) those in which soil was held at different temperatures while the common air temperature was either relatively high or low. In studies of the first type, "dauermycelial" isolates incited severe wilt on tomato plants at 16° and 20° but no wilt occurred at 25°, 27°, and 29° (1, 4), whereas a pseudosclerotial isolate incited wilt at 21.5°, 25°, and 27° but not at 29° (4). In studies of the second type, in which various controlled soil temperatures were combined with a low mean air temperature (20°–21°), a "dauermycelial" isolate incited wilt when soil temperatures were as high as 26° (5) whereas a pseudosclerotial isolate incited severe wilt with soil temperatures at 28° and mild wilt at 31° (6). Thus the disease developed at higher soil temperatures when air temperatures were low than when soil and air temperatures were the same. With high mean air temperatures (31°–32°), disease development was retarded in plants growing in soil at temperatures favorable for disease development (5, 6).

The present study was undertaken to compare the influence of temperature on growth of the 2 types of isolates and to evaluate the influence of soil and air temperatures separately on disease development and on the temperature of the interior of the stem, where the host-parasite interaction occurs.

MATERIALS AND METHODS.—For cultural studies, 3 "dauermycelial" (DM) isolates of the fungus were obtained from potatoes, 2 pseudosclerotial (PS) isolates from cotton, and 1 PS isolate from tomato. The isolates from cotton were found to be nonpathogenic on tomato. Stock cultures were kept on potato-dextrose agar at 4°C. For temperature studies, transfers to petri plates were made with a platinum loop containing sterile water, which was touched to the surface of a 1-week-old culture; the inoculum thus obtained was transferred gently to the center of the dish. To provide inoculum for plants, the fungus was grown on Czapek's solution in Erlenmeyer flasks on a shaker. After 2 weeks, the culture was centrifuged for 10 minutes and the supernatant discarded. The fungus concentrate was suspended in tap water and centrifuged for 10 minutes. The concentrate was resuspended in tap water and brought to the original volume of the culture. The suspension was then homogenized in a Waring Blendor for 1-2 minutes and used for inoculation of plants.

Seed of Bonny Best variety of tomato was sown in vermiculite and watered thereafter with Hoagland's solution. Two weeks later, seedlings were transplanted into quartz sand, 5 to a 6-in. crock. After 16–20 days, plants were pulled and the 4 most uniform plants in each crock selected. The roots were washed and dipped in inoculum for 1–2 minutes and the plants replanted in the original crocks. Control plants were treated likewise except that they were not dipped in inoculum. The crocks containing the plants were placed in Wisconsin soil-temperature tanks in which temperatures were controlled at the desired level. Greenhouse air temperature was controlled by thermostatic regulation of steam radiators and introduction of cold air. Symptoms appeared in 6–7 days, and disease ratings were made in 17–18 days. Plants were placed in 1 of 6 disease classes, and a disease index was calculated for each treatment according to the method of Walker et al (10).

To determine rates of transpiration, plants were transplanted to vermiculite in paraffined paper cartons with paraffined paper covers and watered with Hoagland's solution. Every 2 hours during the experiment, the cartons were removed from the soil temperature tank, wiped with a cloth, and weighed. Transpiration was recorded as grams of water lost per hour. Light intensity was measured with a General Electric light meter. Internal temperatures of plant stems were measured with copper-nichrome thermocouples.

EXPERIMENTAL RESULTS.—*Relation of temperature to fungus growth.*—A comparison of rates of growth on Czapek's agar (pH 7.5) in petri dishes incubated at 16°, 20°, 22°, 24°, 28°, and 32° C was made with 3 PS and 3 DM isolates. Final readings of colony diameter were made at 14 days. The optimum for growth of the 3 DM isolates was 22°. All 3 isolates were slightly retarded at 24°; they grew very slowly at 28°, and no growth was visible at 32°. The optimal growth of the 3 PS isolates was at 24°, although they also grew nearly as rapidly at 22°. They were slightly retarded at 28°, and 2 of the 3 isolates made some growth at 32°. Thus the major difference between the DM and PS isolates was that the latter required a slightly higher temperature for optimal growth than did the former; PS isolates were only slightly retarded at 28°, whereas the DM isolates were distinctly retarded at this temperature.

Relative influence of soil and air temperatures on disease development.—Since the temperature of the soil under natural conditions often differs from air temperature, it seemed logical to evaluate separately the influences of soil and air temperature on disease development. Two experiments were made; the first in March and April, the second in November and December. In each experiment, 12 soil-temperature tanks were used. Each tank represented a different soil-air temperature combination and contained 2 crocks of noninoculated plants and 2 crocks for each of the 3 DM isolates and for the 1 PS isolate pathogenic on tomato. Each crock contained 4 plants. Disease readings were made at 17 days in 1 experiment and at 18 days in the other.

The results in Table 1 are averages of disease indices of 2 replicates in each of 2 experiments. The PS isolate was about equally effective in wilt production at all combinations of air and soil temperature. The 3

TABLE 1.—*Disease and stunting indices of tomato plants inoculated with 1 pseudosclerotial (PS) and 3 "dauermycelial" (DM) isolates of Verticillium albo-atrum while growing at various combinations of air and soil temperatures*

Isolate No.	Isolate Type	Air temperature (°C)	Disease index[a] of plants grown at indicated soil temperature			Stunting index[b] of plants grown at indicated soil temperature		
			20°	24°	28°	20°	24°	28°
P1	DM	16	40	33	7	42	33	8
		20	39	40	7	44	42	8
		24	36	35	6	34	28	11
		28	22	20	0	15	10	5
P33	DM	16	34	37	0	36	36	4
		20	36	31	2	37	28	6
		24	34	32	4	31	29	16
		28	15	21	0	14	10	3
P34	DM	16	36	29	0	36	33	16
		20	36	27	0	37	25	5
		24	31	30	6	22	26	14
		28	19	14	0	19	18	6
T16	PS	16	37	38	39	36	35	31
		20	37	37	44	41	39	37
		24	40	41	46	29	40	37
		28	41	44	44	21	37	38

[a] Based on a scale of 0 to 100, in which 0 indicates no disease and 100 indicates all plants dead. Each index figure is based on disease class values of 16 plants.

[b] The "stunting" index for each isolate and treatment was calculated by dividing the difference between the average height of the inoculated plants and that of the control plants by the latter value and multiplying the dividend by 100.

DM isolates were similar with respect to wilt production. All were about equally pathogenic when air temperatures of 16°, 20°, or 24° were combined with soil temperatures of 20° or 24°. When an air temperature of 28° was combined with soil temperatures of 20° or 24°, the disease was less severe than with the other combinations. When the 16°, 20°, or 24° air temperatures were combined with a soil temperature of 28°, the disease symptoms were slight. When the air and soil temperatures were both at 28°, no symptoms developed. Thus at all air temperatures combined with soil temperatures of 28° and at all soil temperatures combined with air temperatures of 28° there was a marked contrast in wilt index incited by the DM and PS isolates.

The data in Table 1 were submitted to statistical analysis. Since the 2 experiments were conducted at different times of the year, they were considered, for the purpose of analysis, as parts of a split-plot design. Therefore, the error attributable to the fact that the experiments were conducted at different times of the year was taken into consideration in the calculations. The factorial analysis showed that the air temperature effect was statistically significant at the 5 per cent level and that of soil temperature effect was significant at the 1 per cent level. There was no interaction between soil and air temperature, showing that either factor was consistent in its influence at all levels of the other factor.

At the end of each experiment, the height of plants in each treatment was measured. A "stunting" index for each isolate and treatment was calculated by dividing the difference between the average height of the inoculated plants and that of the control plants by the latter value and multiplying the dividend by 100. There was a positive correlation between disease index and stunting (Table 1).

The 2 PS isolates from cotton mentioned previously were nonpathogenic on tomato. When cotton was inoculated with these isolates, they incited severe disease at air and soil temperatures of 28°.

Influence of soil and air temperatures on internal stem temperature.—During the course of the experiments with 12 different soil-air temperature combinations, the question arose as to which of the temperatures (air or soil) was the more influential in determining internal stem temperature. During the day, more water would enter the roots and pass up through the stem than at night because of the higher rate of transpiration during the day. Thus the temperature of the soil water should have a greater influence on the temperature within the stem during the day than during the night. Internal temperature of the stems of noninoculated plants growing in the various soil-air temperature combinations was measured 2, 6, 12, and 18 cm above the soil level during the early afternoon, when transpiration was approaching the maximal level for the day. For the purpose of further discussion, "equivalent temperature level" is defined as that point in the stem above the soil line at which the internal temperature is midway between that of the air and that of the soil. When air and soil temperatures were equal, the internal stem temperature at any point in the stem was practically the same as that of air and soil. When soil temperature was different from that of air, the stem temperature at the soil line was close to that of the soil. When air temperatures were higher than soil temperatures, the internal stem temperature gradually increased as the distance above the soil line increased, although it was always lower than air temperature. When air temperatures were lower than soil temperatures, internal stem temperature gradually decreased as the distance above the soil line increased. Under the conditions of this experiment, the equivalent temperature level was about 12 cm above the soil line in each of the various soil-air temperature combinations in which the 2 temperatures differed.

Relation of transpiration and light intensity to equivalent temperature level.—The next experiment was designed to correlate transpiration and light intensity with changes in internal stem temperature. A combination of 20° (±1°) soil and 28° (±2°) air temperatures was used. The equivalent temperature level in this case was 24°. Stem temperatures were measured at 4, 6, 12, 18, 24, and 30 cm above the soil level in 2 plants about 42 cm tall. For transpiration measurements, 5 plants 10–12 cm tall were used, each in a waxed paper carton that could be weighed at 2-hour intervals on a small triple-beam balance. Incident light intensity was re-

corded hourly during the day.

The cartons were placed in the temperature tank at noon. Stem temperature measurements were begun at 2 p.m. in order to allow the soil temperature to adjust to that of the tank water. Throughout the experiment there was a difference of about 2.5° between the internal stem temperature at 4 cm above the soil level and that at 30 cm (Fig. 1, A). In general, the internal stem temperature was midway between the soil and air temperatures from 2 p.m. to 4 p.m. Between 4 p.m. and 7 p.m., the temperatures of the stem rose toward that of the air and remained at a relatively constant level during the night. Between 5 a.m. and 7 a.m., the stem temperatures began to decline and continued to do so until noon when they were close to the soil temperature.

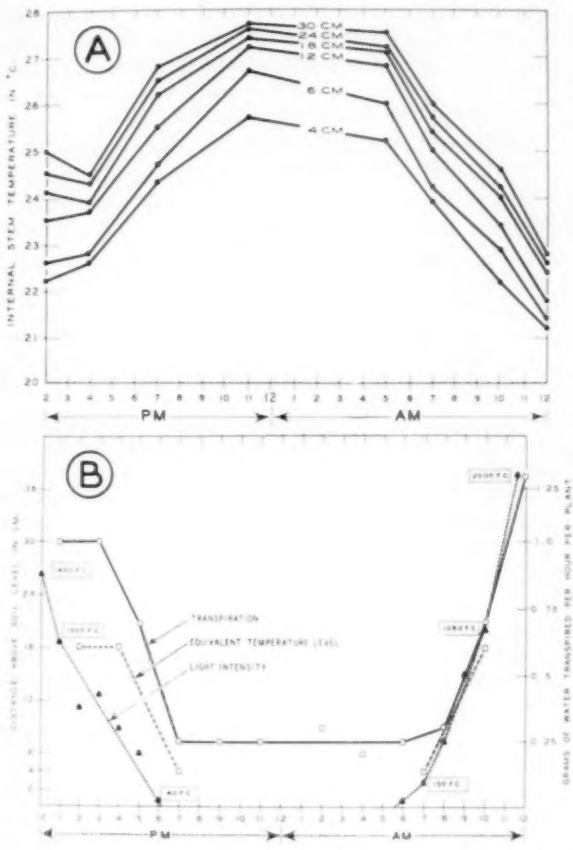

FIG. 1. A) The average diurnal fluctuation of internal stem temperatures of 2 tomato plants about 42 cm tall measured at 4, 6, 12, 18, 24, and 30 cm above the soil level, with air temperature of 28°C (±2°) and soil temperature of 20° (±1°). B) Relation of transpiration and light intensity to the equivalent temperature level in tomato plants grown in soil at 20°C(±1°) and air at 28° (±2°). The values of equivalent temperature level, which is the height above the soil line at which the internal stem temperature is midway between that of the air and that of the soil (in this case 24°), were calculated from the data in Figure 1,A. The transpiration data were obtained by weighing, at 2-hour intervals, 5 plants 10–12 cm tall growing in waxed paper cartons. Light intensity during the day was measured in foot-candles with a General Electric light meter.

The equivalent temperature level curve shown in Figure 1, B is based on values calculated from the data in Figure 1, A. In the same figure are presented the transpiration and light intensity curves for the same period. The equivalent temperature level, i.e. the point at which the internal stem temperature was 24°, was 18 cm at 10 a.m., 2 p.m., and 4 p.m. Between 10 a.m. and 2 p.m., the internal stem temperature, at all points measured, was below 24°. After 4 p.m., the equivalent temperature level gradually declined. After 7 p.m. and during the night until 7 a.m., the equivalent temperature level was below 4 cm. Since measurements were not made below 4 cm it was not possible to calculate the equivalent temperature levels for the night period. After 7 a.m., the curve rose rapidly to 18 cm at 10 a.m.

There was a close correlation between light intensity, transpiration, and equivalent temperature level. When soil temperature is lower than air temperature, as was the case in this experiment, the former has a greater influence on the interal stem temperature during the day than during the night.

DISCUSSION.—The higher optimal and maximal temperatures for growth in culture of the PS isolates over those of DM isolates is in agreement with previous reports (1, 2, 4, 5, 6). Although previous studies have indicated that both air and soil temperatures influence disease development, no attempts have been made to evaluate the relative influence of air and soil temperatures. In these studies, both of these temperatures were found to influence disease significantly, with soil temperature being the more influential. This explains why high or low mean air temperatures have altered the results obtained previously when only soil temperature was controlled (5, 6).

When the relative effects of soil and air temperatures on internal stem temperature were evaluated over a 24-hour period, the former was found to be the more influential for about 6 hours during the day, whereas the latter was the more so during the night. In the upper part of the stem the temperature was closer to air temperature than to soil temperature for the majority of time, yet soil temperature was more effective than air temperature in influencing disease severity. It would seem, therefore, that the center of greatest activity of the fungus must have been in the roots and lower stem under the conditions provided in these experiments. This is to be expected, since penetration is through the roots and the symptoms first appear on the lower leaves, the vascular traces of which extend into the hypocotyl. As the plants grow taller and the fungus advances farther up the stem, it would be expected that air temperature would become more influential.

The cyclic influence of soil and air temperatures on stem temperature probably can be explained on the basis of differences in rate of water uptake by roots during the day and during the night. During the day, when transpirational rate is high, the temperature of the soil water absorbed by the roots and transported

upward through the xylem has a dominant influence on the stem temperature, whereas at night water uptake is slow and therefore has little influence on stem temperature. The rate of transpiration showed a general positive correlation with light intensity. The maximal light intensity measured in these studies was 2000 foot-candles. The situation out-of-doors would be quite different, with light intensities attaining 10,000 foot-candles or more on bright days. Studies with leaves of numerous species, including cactus plants and trees, have shown that plant parts may be heated by isolation to temperatures several degrees higher than the surrounding air (3). Thus, Waggoner and Shaw (9) measured stem temperatures of tomato plants on a bright August day in Iowa and found that the temperature in a partially shaded stem was about 12° F cooler than was the surrounding air; in an unshaded stem, the temperature was very close to that of the surrounding air. This would indicate that the transpirational stream was cooling the stem, soil water being cooler than air in midday. With the shaded stem, the influence of the transpirational stream might have been the dominant factor; with the unshaded stem, the process of insolation might have counteracted completely the cooling effect of the transpirational stream. The data from the current investigation are too incomplete to make any evaluation of the 2 different processes at any given light intensity. In these studies, with a maximal light intensity of 2000 foot-candles, the effect of insolation on stem temperature was relatively small because the internal stem temperature became cooler as light intensity increased.

Another factor that could influence internal stem temperature is radiation from the plant to the cold soil, which would cause some cooling of the plant. During the day, radiation from the plant surface keeps the plant from being damaged by insolation (3). During the night, radiation from the plant would tend to keep it cooler than the surrounding air. Since some water uptake occurred at night however, it was not possible to evaluate the relative cooling effect of radiation from the plant and that of water uptake by the roots.

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POWDERY MILDEW PIMPLES ON WATERMELON FRUITS[1]

S. S. Ivanoff

SUMMARY

Hyperplastic symptoms on greenhouse-grown watermelon fruits, in the form of pimples, warts, and similar intumescences, were found to be caused by a species of the family *Erysiphaceae*, probably *Erysiphe cichoracearum* DC. The spots infected by the powdery mildew fungus were very similar to those occurring on leaves of cantaloupes, whereas the intumescences resembled pimples and other excrescences previously observed on watermelon fruits and found associated with a strain of tobacco ringspot virus or due to some unknown cause. A preliminary test has shown that fungicidal dips may be of help should this disease prove to be of economic importance.

Hyperplastic symptoms in the form of "pimples" or "warts" were found associated with, and originating from, infection by a powdery mildew fungus on young watermelon fruits in the greenhouse. Beneath the areas where fungus growth had been, various abnormal excrescences or intumescences of various sizes appeared on the unbroken rind (Fig. 1, 2).

Hyperplastic abnormalities of uncertain origin have been known by watermelon growers and plant pathologists for some time and have been commonly referred to as pimples, measles, sand blisters, gum pockets, bumps, and other names.[2] Recently, pimples on watermelon fruits have been reported to be associated with a strain of tobacco ringspot virus.[3, 4]

Pimples (cause unknown) on watermelons were brought to the writer's attention by shippers in Texas during the 1942-43 seasons when such abnormalities were very prevalent. Many loaded cars were abandoned because of "leakage," which proceeded from disintegrating, soft-rotting pimples on the bottom side of fruits. A hole, sometimes of the diameter of a pencil, frequently formed through the rotting pimple. The disease was confused with anthracnose. Isolations from intact pimples yielded no fungus or bacterial colonies on nutrient agar.

Similar pimples, still of undetermined etiology, were observed by this writer in June, 1956, near Lucedale, Mississippi, in a sheltered field of several acres of Black Diamond watermelons. Every fruit seen in that field had these pimples.

Powdery mildew on watermelon leaves and fruits has been reported from various places in the United States and from abroad,[5] but no association or causal relationship between mildew and pimples, or any other hyperplastic symptom, has been previously reported, as far as is known to this investigator.

Powdery mildew appeared on young greenhouse-grown fruits soon after they were formed. The infected areas or spots resembled common powdery mildew spots on cantaloupe vines that were growing at that time in an adjacent bench. (Some young cantaloupe leaves showed atypical small round puckerings and thickenings on the leaf surface where small colonies of the pathogen had originated.) No powdery mildew was seen at any time on the leaves of the watermelon vines. Microscopic examination of the fungus growth from the fruits showed it to belong to the true powdery mildew group of fungi, *Erysiphaceae*. The conidia were oval hyaline single cells produced in chains on the ends of erect unbranched conidiophores. The mycelium was typical of the *Erysiphaceae* and covered the infected spot in a web-like fashion. Further identification of the causal organism would require an examination of the ascigerous stage. It is likely, however, that the disease was caused by *Erysiphe cichoracearum* DC, inoculum of which was present in abundance since before the time the watermelon fruits began to form.

From 7 to 10 days following the appearance of the mildew spots on the fruit, the area covered by the fungus growth had become more or less raised. The borders of the raised area followed almost exactly the outline of the mildew spot, and the resulting wart or "pimple" was well circumscribed (Fig. 1, A, B). The fungus growth seemed to disappear even before the intumescences were fully formed. The surfaces of the pimples were either smooth or rough. In some cases these pimples were of a symmetrical conical shape. Their size at the base varied from 1 to several millimeters in diameter. Usually there was only a single pimple formed underneath a small mildew spot; in other cases there were numerous small ones beneath a large mildew area (Fig. 1, B, at left). In some cases, apparently under conditions of low humidity, the affected areas became corky and gave the appearance of "russetting." These areas often were quite extensive, solidly covering up to 20 per cent of the fruit surface. When fruits with such manifestations were returned to an atmosphere of high humidity, however, the fungus resumed its growth around the circumscribed periphery of the russetted area, and secondary intumescences of greater prominence eventually were formed in a ring around the original infected spot (Fig. 2, A). The

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[4] Shepherd, R. J., and F. B. Struble. 1956. Tobacco ringspot virus on watermelons. Phytopathology 46: 358-362.

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epidermis of a pimple remained unchanged for some time, but with age and exposure to dry atmosphere the

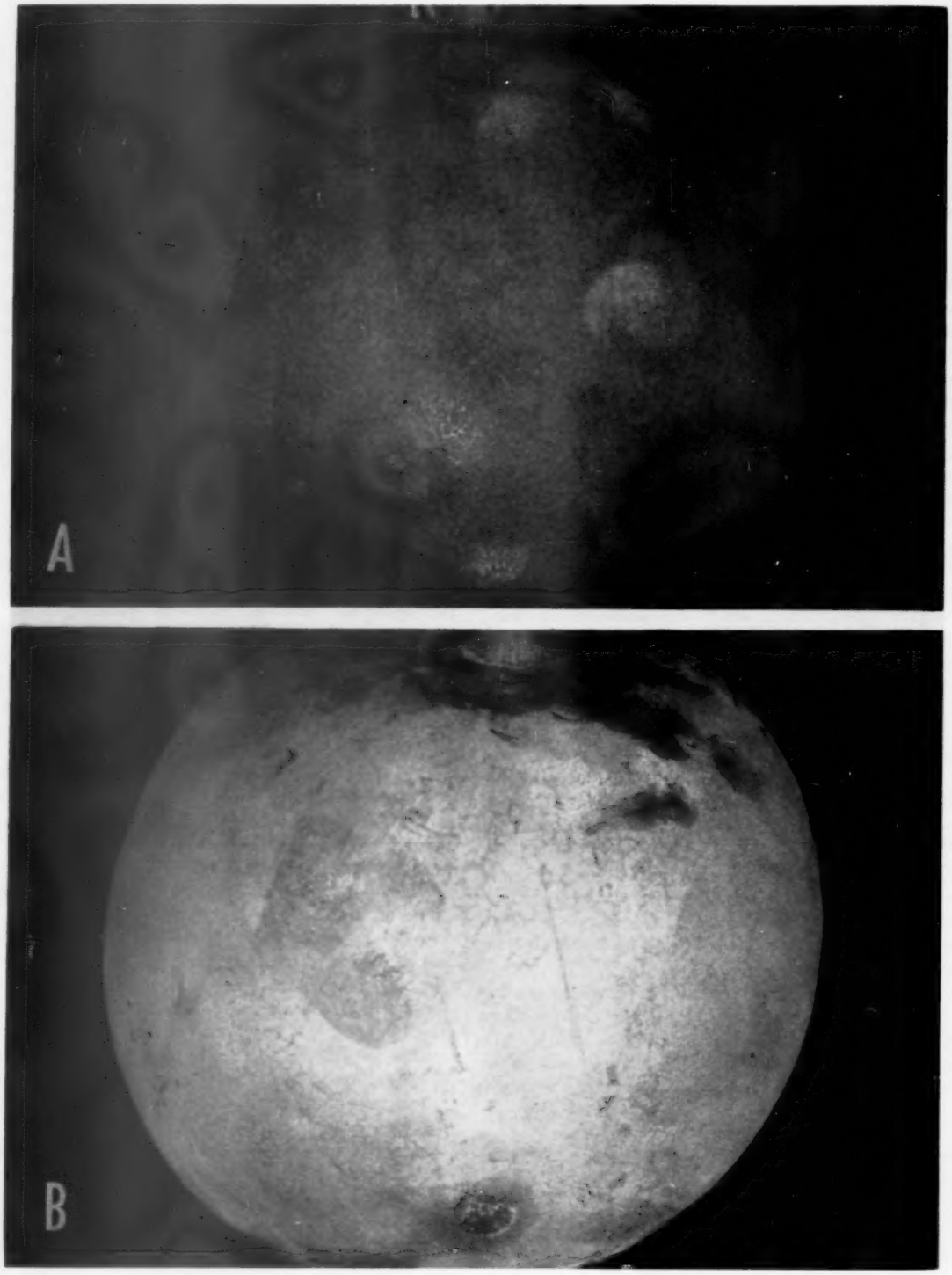

FIG 1. Powdery mildew and powdery mildew pimples on watermelon fruits (approximately natural size). A) Young fruit with several powdery mildew spots and the beginning of the appearance of pimples. B) Same fruit a few days later, showing well-formed pimples at the places where the mildew spots had been. A single large pimple is shown near the blossom end with groups of small pimples directly above and also to the left. After a time, necrosis may develop on the latter and give the area the appearance of russetting.

surface became necrotic, fissured, or wrinkled, either in part or entirely (Fig. 2, B).

The number of fresh infected spots on a single fruit varied from a few to over a hundred, and intumescences

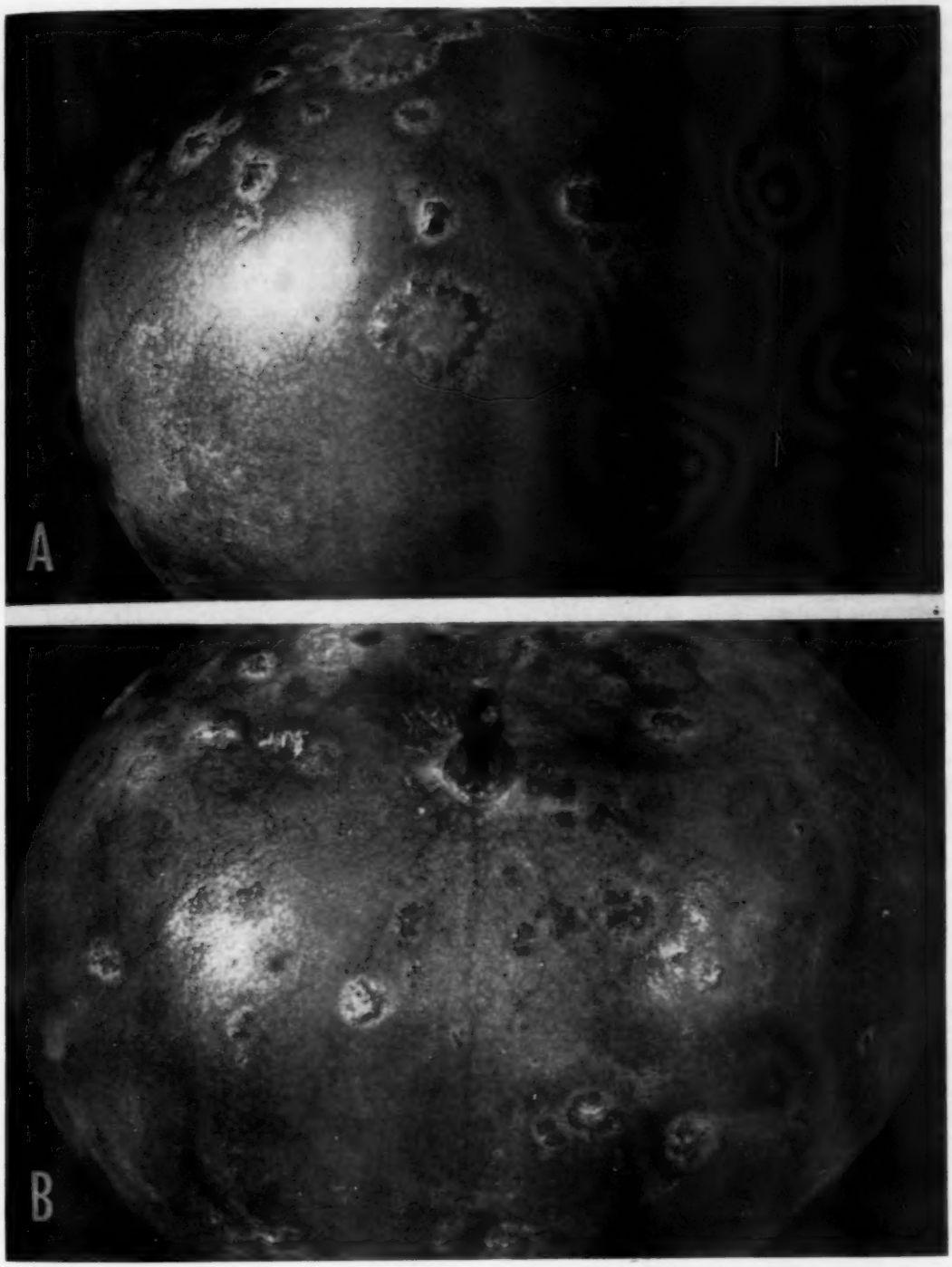

FIG. 2. Powdery mildew pimples and other intumescences on young watermelon fruits (approximately natural size). A) Large intumescence (at meridian position) consisting of 2 parts: 1) a relatively flat, necrotic "russetted" area induced by a large primary powdery mildew fungus colony at low humidity; and 2) a ring of intumescent formations produced later around the rusetted area upon the return of the fruit to high humidity. Other types of intumescences also are shown. B) Several old pimples (near bottom right) showing necrotic fissured surface tissue. When newly formed, these pimples had smooth green surfaces.

appeared under each such spot. When the mildew spots coalesced at places, the intumescences underneath them also coalesced. On a number of fruits hyperplasia occurred all over the circumference, including the bottom side of the fruit. There were no intumescences where there was no preceding growth of the powdery mildew pathogen.

The mildew spots were examined closely for the presence of mites or insects that might possibly be associated with the hyperplastic growth amid a special environment, but none were found.

Approximately 35 fruits of all sizes, from 9 vines of crosses between Black Diamond type watermelons, were studied during a period of 3 months. All young or mature fruits, except some of those given the treatments mentioned below, developed mildew and pimples.

Dipping young fruits in a suspension of Karathane[6] (1:2000, with a small amount of Triton B-1956[7] added) prevented infection by the powdery mildew fungus and also prevented hyperplasia. It also eradicated fungus growth on some fruits. By this treatment, pimples were prevented from forming under newly formed mildew spots, but they did form on some fruits where the pathogen had already reached the sporulating stage at the time of the treatment. This preliminary test suggests a method of practical field control that could be used should this disease prove to be of economic importance.

The relationship between the nature of the powdery mildew pimples here reported and the pimples of virus association or origin, as well as other similar abnormalities previously described, needs further investigation and clarification. For the purpose of classification, however, it is proposed that the above-described hyperplastic formations be referred to as powdery mildew pimples.

[6] A Rohm and Haas product containing 22.5 per cent 2-(1-methylheptyl)-4,6-dinitrophenyl crotonate and 2.5 per cent isomers.

[7] Modified phthalic glycerol alkyd resin A (Rohm and Haas).

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EFFECTS OF SOME PHENOLS AND QUINONES ON GROWTH IN VITRO OF VERTICILLIUM ALBO-ATRUM[1]

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SUMMARY

The effects of 13 phenols and phenolic acids and 4 quinones on the growth in a liquid medium of *Verticillium albo-atrum* Reinke & Berth. were determined. Prior to inoculation, the test materials were added to a sucrose-salts medium, and the mycelial mats were harvested at regular intervals. Of the 3 isomeric dihydroxybenzenes, catechol (with hydroxyl groups in the *ortho* position) was most inhibitory. Replacement of a hydroxyl group with a carboxyl group or the introduction of a carboxyl group on the ring decreased the effectiveness of catechol. Pyrogallol, which has 3 hydroxyl groups on adjacent carbon atoms, was the most toxic phenolic compound tested. The quinones were generally more inhibitory than were the phenols to *V. alba-atrum*. Also, 1,4-naphthoquinone was more inhibitory than 1,4-benzoquinone. Chlorination of the quinones increased their effectiveness as inhibitors. The compound 2,3-dichloro-1,4-naphthoquinone caused considerable inhibition at 10^{-6} M (less than 1 ppm).

Many workers have implicated phenols or tannins as possible causes of disease resistance in plants (15). The most notable example in the earlier literature is the work of Link and coworkers (5, 6), who showed that colored onion varieties resistant to *Colletotrichum circinans* (Berk.) Vogl. contained more catechol and protocatechuic acid in their bulb scales than did less resistant varieties. Walker and Link (14) found that these 2 compounds were toxic to the organism *in vitro*. Their tests also showed that the toxicity of other phenolic compounds to *C. circinans* and to 3 other fungi was dependent on the molecular structure of the phenol (14).

Recently, Schaal et al (12) reported that the periderm of tubers of potato varieties resistant to the scab organism *Streptomyces scabies* (Thaxt.) Waks. & Henrici gave a deeper green coloration with ferric chloride than did tubers of susceptible varieties. This differ-

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ential green reaction with ferric chloride, the result of reaction with *o*-dihydroxy groups, was shown to be due primarily to the presence of chlorogenic acid (3). More recently, it was shown that this compound and materials related to it are inhibitory to the growth *in vitro* of the scab organism (13).

During the testing of tubers of a large number of potato (*Solanum tuberosum* L.) varieties and selections with ferric chloride, it became apparent that the intensity of the reaction was also correlated with Verticillium wilt resistance (7). This led to investigation of the distribution of chlorogenic acid within other parts of the potato plant and of the effect of chlorogenic acid on *V. albo-atrum* (Lee and Le Tourneau, unpublished results). In conjunction with these investigations, experiments were made to determine the effect of other phenolic and related compounds on the growth of *V. albo-atrum* in culture. The results of these experiments were reported briefly in an abstract (5).

MATERIALS AND METHODS.—The experiments in liquid culture were made with a synthetic medium containing the following amounts of major salts and sugar per liter of medium: $MgSO_4 \cdot 7H_2O$—0.5 g; KCl—0.5 g; KH_2PO_4—1.0 g; $NaNO_3$—3.0 g; and sucrose—30 g. An aliquot of a minor element solution was added so that the final concentrations per liter were as follows: molybdenum and manganese—0.02 mg; copper and zinc—0.1 mg; and iron—0.2 mg. Manganese, copper, and zinc were added as sulfates, iron as ferric nitrate, and molybdenum as sodium molybdate. Following the addition of the calculated amount of material to be tested, a medium was adjusted to pH 4.5 and dispensed (25 ml per 125-ml Erlenmeyer flask). The flasks were plugged with cotton and autoclaved 15 minutes at a pressure of 15 lb. Because of their insolubility in water, tetrachlorobenzoquinone, 1,4-naphthoquinone, and 2,3-dichloro-1,4-naphthoquinone were first dissolved in acetone; 1 ml of acetone containing the desired amount of quinone then was added to each flask. Tetrachlorobenzoquinone and 2,3-dichloro-1,4-naphthoquinone were furnished by the United States Rubber Co. All other compounds were Eastman White Label or of comparable purity.

Inoculum for an experiment was prepared by seeding several control flasks of the synthetic medium with a small mycelial biscuit from a culture growing on potato-dextrose agar. Following this, the flask was shaken briefly and set aside. In 2 or 3 days the conidia had germinated, and many small colonies were barely visible. One flask was selected and 1 loop of this suspension was used for each of the flasks in the test series. Thus the amount used for seeding was constant for any 1 experiment, but it was not standardized from experiment to experiment. The use of this mycelial suspension eliminated some of the lag in growth obtained when a spore suspension was used.

The seeded flasks were arranged at random on the laboratory bench at room temperature (21°–25°C). Three flasks of each treatment were harvested at 4-to 6-day intervals. The pH of the medium at the time of harvest was determined with a glass electrode (Beckman Model H-2). The mycelial mats were filtered on Gooch crucibles lined with asbestos or on sintered-glass crucibles. The mats were washed gently 2 or 3 times with water to remove adhering salts and sugar and then dried overnight at 90°–95°C.

Assays were made also with penicylinders placed on agar media. The liquid media above was solidified with 2 per cent agar and dispensed, 25 ml per petri plate. After the agar had solidified, sterile penicylinders were placed in the surface of the agar. The plates were seeded with 1.5 ml of a spore suspension obtained by flooding the surface of a 10- to 14-day-old culture with sterile distilled water. After the plates were swirled to insure uniform spore distribution, the excess liquid was poured off and 0.1 ml of the desired phenol solution added to each penicylinder. Phenolic solutions were adjusted to pH 4.5 and prepared fresh for each test. The plates were incubated 48–72 hours and the zone of inhibition measured in millimeters.

Experiments in liquid culture were made primarily with 1 isolate designated 4B. This isolate is a white mycelial type that does not normally produce microsclerotia when cultured on synthetic media. Two additional isolates were used in the penicylinder tests.

RESULTS.—Initial experiments were made with the *o*-dihydroxy compound, catechol. Above approximately 150 ppm, catechol was completely inhibitory. Figure 1,A summarizes an experiment in which 50, 100, and 150 ppm of catechol were added to basal medium. The addition of catechol to the medium also stimulated the production of microsclerotia. During the growth of *V. albo-atrum*, the pH of the medium became progressively more alkaline (Fig. 1, B). The change in pH was related to the amount of growth, since rapid changes were not observed when growth was inhibited. In further experiments, 10^{-3} M catechol was chosen as a standard for comparing the inhibition caused by other phenolic compounds.

The comparative effects of the 3 isomeric dihydroxybenzene compounds at 10^{-3} M are shown in Figure 1, C. The *meta* isomer, resorcinol, had no effect on fungus growth. The *para* isomer, hyroquinone, reduced growth considerably but was not as effective as catechol. These results agreed with those of Walker and Link (14), who worked with *Colletotrichum circinans*, *Gibberella saubinetii* (Mont.) Sacc., *Botrytis allii* Munn, and *Aspergillus niger* v. Tiegh. Greathouse and Rigler (1), however, found that hydroquinone was more toxic toward *Phymatotrichum omnivorum* (Shear) Duggar than was catechol.

The results with the 3 isomeric trihydroxybenzene compounds especially illustrate the effects of the structure of a compound on fungus growth. Phloroglucinol (1,3,5-trihydroxybenzene) had no effect on the growth of *V. albo-atrum* at 10^{-3} M. At this concentration, hydroxyhydroquinone (1,2,4-trihydroxybenzene) completely inhibited growth. Hydroxyhydroquinone was still effective in suppressing growth at 2×10^{-4} M. Pyrogallol (1,2,3-trihydroxybenzene) was by far the

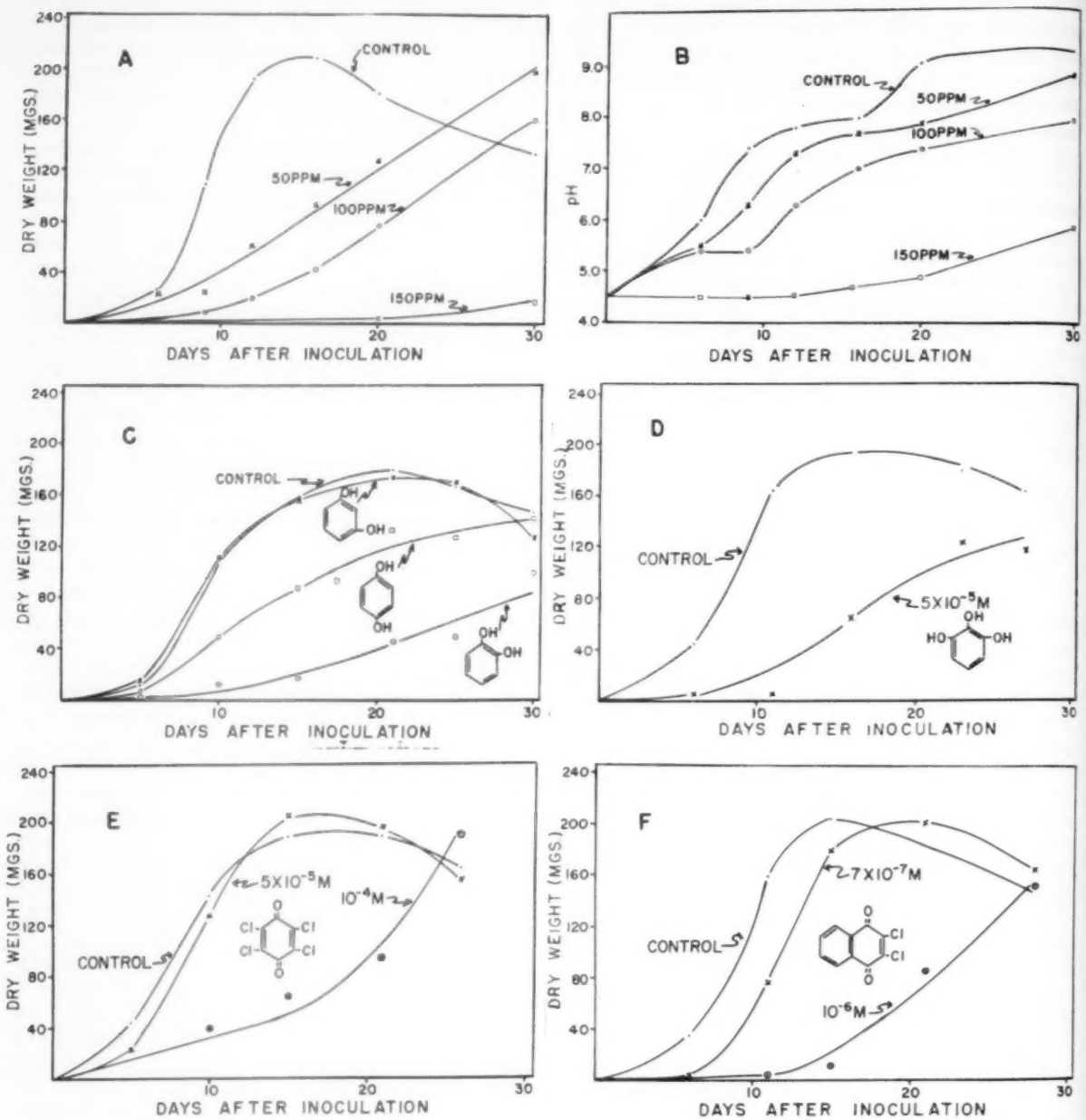

FIG. 1. Effects of various phenols and quinones on growth of *Verticillium albo-atrum:* a) 50, 100, and 150 ppm of catechol; b) pH changes in medium containing 50, 100, and 150 ppm of catechol; c) 10^{-3} M catechol, resorcinol, and hydroquinone; d) 5×10^{-5} M pyrogallol; e) 10^{-4} and 5×10^{-5} M tetrachloro-*p*-benzoquinone; f) 10^{-6} and 7×10^{-7} M 2,3-dichloro-1,4-naphthoquinone.

most inhibitory. This compound, containing 3 hydroxyl groups on adjacent carbon atoms, was completely inhibitory at 1×10^{-4} M. The results of an experiment with 5×10^{-5} M pyrogallol are given in Figure 1. The toxicity of this compound to other fungi has been reported by others (1, 14).

It is possible that derivatives of pyrogallol might be still more effective. Chlorination of a phenol often enhances fungicidal activity (2). On the contrary, the addition of certain groups to the pyrogallol nucleus could render it less inhibitory. It was found in the present study that the addition of a carboxyl group in position 5 of pyrogallol to form gallic acid greatly decreased its inhibition of *V. albo-atrum*. At 10^{-3} M, gallic acid caused slight inhibition only during the early phases of growth and was less effective than catechol.

Figure 2 lists the structural formulas of catechol

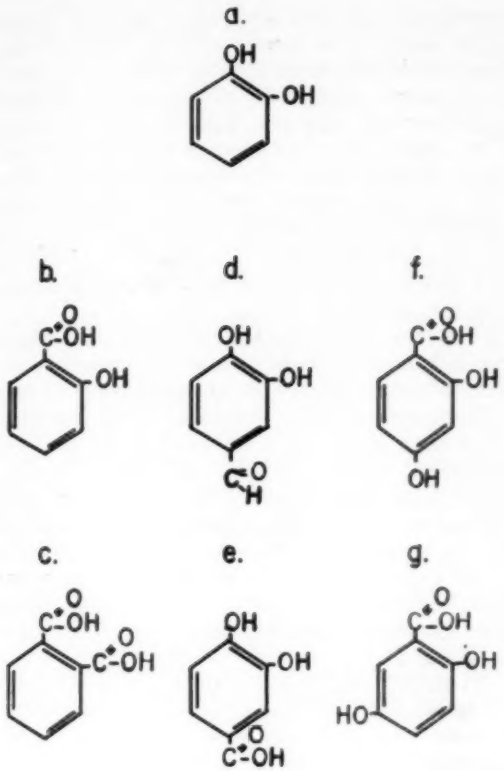

FIG. 2. The structural formulas of catechol and some compounds less effective than catechol in inhibiting the growth of *Verticillium albo-atrum*. a) Catechol. b) Salicylic acid. c) *o*-Phthalic acid. d) Protocatechuicaldehyde. e) Protocatechuic acid. f) 2,4-Dihydroxybenzoic acid. g) 2,5-Dihydroxybenzoic acid.

and several compounds that do not inhibit or are only weakly inhibitory to the growth of *V. albo-atrum*. The replacement of 1 hydroxyl group of catechol to form salicylic acid (Fig. 2,b) greatly reduced its toxicity to *V. albo-atrum*. Slight inhibition with 10^{-3} M salicylic acid was noted at only the first and second harvests. Phthalic acid (Fig. 2,c), in which both hydroxyl groups are replaced by carboxyl groups, did not inhibit growth at 10^{-3} M.

Four trisubstituted benzene derivatives containing 2 hydroxyl groups and 1 other group were investigated. Protocatechuic acid (Fig. 2,e) did not inhibit growth at 10^{-3} M. This compound is partially responsible for resistance of onions to *Colletotrichum circinans* (7). Protocatechuic aldehyde (Fig. 2,d) caused inhibition at 10^{-3} M during the early phases of growth, but it was less inhibitory than catechol. Neither 2,4-dihydroxybenzoic acid nor 2,5-dihydroxybenzoic acid (Fig. 2,f,g) inhibited growth of *V. albo-atrum* at 10^{-3} M.

In general, quinones are often more effective inhibitors of fungi than are the corresponding phenols. It has been suggested that the inhibitory effects of phenols may be due to their conversion to quinones (8, 10). Schaal and Johnson (13) suggested this possibility to explain the resistance of potato tubers to *Streptomyces scabies*.

To assess the inhibitory effect of quinones on *V. albo-atrum*, experiments were made with 4 compounds. *p*-Benzoquinone completely inhibited growth at 5×10^{-4} M. Further experiments showed considerable inhibition at 2×10^{-4} M, whereas a concentration of 5×10^{-5} M no longer inhibited the fungus. Except for pyrogallol, *p*-benzoquinone was more inhibitory than any of the phenols tested. It is to be noted that *p*-benzoquinone was much more inhibitory than its parent phenol, hydroquinone.

The addition of 4 chlorine atoms to *p*-benzoquinone to form tetrachloro-*p*-benzoquinone changed its toxicity only slightly (Fig. 1,E). Although tetrachloro-*p*-benzoquinone may inhibit at concentrations lower than does *p*-benzoquinone, its inhibitory action decreases with decreasing concentration more rapidly than does that of *p*-benzoquinone. Generally, chlorination increases the fungitoxicity of quinones (8). It is possible that in this study the tetrachloro-*p*-benzoquinone may have decomposed during the course of the experiment because of its prolonged exposure to light.

The compound 1,4-naphthoquinone proved to be more inhibitory than the corresponding benzoquinone. At 2×10^{-5}M there was no measurable growth, whereas 10^{-5} M caused considerable inhibition. Inhibition was still detectable at 5×10^{-6} M.

The addition of 2 chlorine atoms to 1,4-naphthoquinone to form 2,3-dichloro-1,4-naphthoquinone resulted in additional inhibition to fungus growth. Growth was completely inhibited by 2×10^{-6} M. At a concentration of 10^{-6} M, there was some growth and concentrations as low as 7×10^{-7} M still resulted in measurable inhibition in the early phases of growth (Fig. 1,F).

Several tests showed that the same general results were obtained whether the medium was sterilized by autoclaving or Seitz filtration. Nevertheless it seemed desirable to determine the effects of these same compounds when chances for degradation or interaction with other components of the medium were minimized. For this purpose, assays were made with penicylinders placed on agar medium.

Most of the compounds investigated in liquid medium were also tested against 2 additional isolates in the penicylinder assays. The 3 quinones tetrachloro-*p*-

TABLE 1.—*Penicylinder assays of the inhibitory effect of 2 phenols and 1 quinone against 3 isolates of Verticillium albo-atrum*

Chemical	Amount of chemical per penicylinder (mg)	Inhibition zone (mm) of indicated isolate of *V. albo-atrum* 4B	16B	2
Catechol	1.1	37	38	43
Pyrogallol	0.13	37	43	41
Pyrogallol	0.26	55	46	54
p-Benzoquinone	0.11	37	37	34
p-Benzoquinone	0.22	50	54	41

benzoquinone,1,4-naphthoquinone, and 2,3-dichloro-1,4-naphthoquinone are not soluble in water. Results with these compounds were erratic and of doubtful value and will not be considered in the discussion.

Results of penicylinder assays with 3 compounds are given in Table 1. This experiment, as well as others, showed that results obtained on a solid medium compared favorably with those in a liquid medium. In some cases it appeared that there might be differences among the isolates in their response to the chemicals. It would be necessary, however, to investigate these differences further before any conclusions could be made.

Discussion.—The results of these experiments show that the toxicity of a phenol or of a quinone to *V. albo-atrum* is dependent on the arrangement of the groups on the ring system. Generally, the results obtained with this fungus are comparable with those obtained with other fungi growing on liquid media or with spore germination tests (1, 10, 14).

The reason for the pronounced specificity of certain compounds is not fully understood. Various suggestions have been made to explain the mechanism of action of phenols and quinones against fungi (1, 8), including recent attempts to explain their action through effects on enzymes (9, 10, 11). Even as enzyme inhibitors these compounds might have multiple effects depending on the particular organism involved. Owens (9) pointed out that there are at least 3 different possible interactions (with sulfhydryl groups, with amino groups, and with metals required for enzyme action) of phenols and quinones with enzymes. Regardless of the mechanism, the goal of these tests was to provide information to assist in controlling a disease in the field. Effects *in vitro* are far removed from field conditions where an additional factor, the host plant, is involved. This raises such problems as the phytotoxicity of the compound and its penetration, translocation, and localization within the plant.

In the case of Verticillium wilt, the more effective compounds might be used as a seed-piece treatment to inhibit infection or as a foliar application to retard the development of the disease in the vascular system of the potato plant. Both approaches are now being investigated. Young (16), in work with potatoes in Oregon, noted some reduction in Verticillium wilt by treating seed pieces with 2,3-dichloro-1,4-naphthoquinone. In the present study, trials during the 1955 and 1956 growing seasons indicated that foliar applications of certain phenolic compounds delayed the appearance of wilt symptoms.

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EFFECT OF CERTAIN FUNGICIDES ON SCLEROTIUM ROLFSII IN THE SOIL [1]

D. V. W. Abeygunawardena and R. K. S. Wood

SUMMARY

A number of fungicides were added to soil in which viable sclerotia of *Sclerotium rolfsii* had been buried. When either formalin or D-D, a mixture of dichloropropene and dichloropropane, was added as a fumigant to soil below the sclerotia, the sclerotia were not killed. Neither were they killed when either SR 406 (50% captan) or sodium dehydroacetate was added to the surface of the soil as a drench. In contrast, all or almost all of the sclerotia were killed when either chlorobromopropene or chloropicrin was used as a fumigant or when formalin was used as a drench.

Soil was infested with mycelium of the pathogen, and the amount of viable mycelium after various treatments was assessed from counts of the number of healthy seedlings emerging after sugar beet seed had been sown in the soil. Excellent control of disease was obtained when sodium dehydroacetate, captan, or formalin was used as a drench, but the growth of seedlings following treatment with captan was considerably retarded. Chloropicrin as a fumigant was as good as these soil drenches. Chlorobromopropene probably killed most of the mycelium in the soil, but enough of the chemical was retained by the soil after 2 weeks of aeration to affect emergence and to affect growth of the seedlings that did emerge.

Captan was also tested as a dry dust well mixed with infested soil; very good control of disease was obtained, and there was very little phytotoxicity. Pentachloronitrobenzene behaved similarly, but tetrachloronitrobenzene was not effective except at a rate at which it was toxic to the seedlings.

The fungus *Sclerotium rolfsii* Sacc. is an important parasite of many plants in tropical and subtropical regions. Typically it is soil borne and attacks plants at or about soil level; large numbers of sclerotia generally are produced in infected tissue and on the surface of the surrounding soil. There can be little doubt that these sclerotia are important for the persistence of the fungus in the soil in the absence of suitable host plants and in unfavourable environmental conditions. The elimination of the pathogen from infested soil therefore necessitates the killing of sclerotia as well as the mycelium that may be present.

In recent years, a number of workers have studied the effect of certain fungicides on sclerotia in pure culture or on the fungus in the soil. Davey and Leach (1) used formalin, carbon disulphide, and chloropicrin successfully against sclerotia buried in soil; Wolf (7) suggested that dehydroacetic acid might be used to control the pathogen, and Kreutzer and Montagne (5) and Haasis (3) have shown that chlorobromopropene in small doses killed sclerotia in infested soil. Martin (6) obtained promising results with the fumigants Dowfume W-85 (83% 1,2-dibromoethane) and D-D mixture. Gould (2) found that attack of bulbous iris by *S. rolfsii* was increased when either D-D mixture or chlorobromopropene was added to infested soil and decreased when pentachloronitrobenzene was added. In this paper are reported the results of preliminary experiments in which a number of commercially available fungicides were tested for their ability to kill sclerotia in soil and to control disease under greenhouse conditions.

MATERIALS AND METHODS.—The isolate of *S. rolfsii* that was used was originally obtained from diseased tomato plants in Ceylon; it was reisolated after passage through a tomato plant and maintained on a glucose-peptone agar.

Sclerotia were obtained by growing the fungus on a medium consisting of maize meal and sand in the proportions by weight of 2:98 and containing sufficient water to saturate the mixture; the fungus grew well on this medium and produced large numbers of sclerotia during 3 weeks' growth at 25° C. The sclerotia were readily separated from the rest of the culture and were uniform in shape and size, being spherical and about 2 mm in diameter. Before use, they were surface sterilized by soaking for 5 minutes in a solution containing 0.25 per cent by volume of a saturated solution of bromine in water; they were then washed in several changes of sterile distilled water and dried on sterile filter paper at room temperature.

Soil was contaminated with the pathogen by adding sand-maize cultures in which sclerotia had not yet been produced. The cultures were passed through a 3-mm mesh sieve and mixed with John Innes potting compost in the proportion 1:4 by weight; 200 g of this mixture was spread evenly over the surface of 800 g of soil in tins 5 in. deep and 6 in. in diameter.

The soil used was a fertile medium loam with a water-holding capacity of 44 per cent (100 g dry soil + 44 g water = saturated soil) and a pH of 6.5. It was passed through a 4-mm sieve and the water content was adjusted to 75 per cent of saturation before use.

The fungicides used were as follows: chlorobromopropene (1-chloro-3-bromopropene), chloropicrin (trichloronitromethane), D-D mixture (1,3-dichloropropene; 1,2-dichloropropane), sodium dehydroacetate, SR 406 (50% captan, which is *N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide, plus 48% clay and 2% wetting agent), TCNB (20% tetrachloro-*p*-nitrobenzene, 80% kaolin), PCNB (20% pentachloronitrobenzene, 80% kaolin), and formalin (40% formaldehyde in water). Details of the ways in which these materials were used together with other methods will be given later.

[1] Accepted for publication May 8, 1957.

TABLE 1.—*Germination of sclerotia of Sclerotium rolfsii following treatment (in soil) with various fumigants*

Fumigant [a]	Percentage germination of sclerotia [b]	
	Uncovered soil	Covered soil
D-D mixture	100	0
Chlorobromopropene	0	0
Chloropicrin	17	7
Formalin	100	100
Control (water)	100	100

[a] One ml per 1200 g soil.
[b] Each figure is the mean of 2 tests, each with 50 sclerotia per treatment.

EFFECT OF FUNGICIDES ON SCLEROTIA IN THE SOIL.—*Different fumigants.*—The soil was placed to a depth of 3 in. in the tins; glass cylinders 25 mm long and 10 mm in diameter (internal) were pressed into the soil at the centre to a depth of 12.5 mm, and 50 air-dried sclerotia were placed in the upper half. More soil was added to a final depth of 4.5 in.; the same weight (1200 g) of soil was placed in each tin. Half of the tins were covered with cellophane, the others were left uncovered. One ml of the fumigant was injected from a hypodermic syringe into the soil 2 in. below the sclerotia. The opening in the side of the tin through which the needle was inserted was sealed immediately, and the needle was withdrawn. After treatment, the tins were placed in well-ventilated frames in which the temperature varied between 17° and 26° C during the experiment. The moisture levels of the soil in the uncovered tins were adjusted periodically to the original values by spraying the surfaces with water. Each treatment was in duplicate.

After 4 days, the sclerotia were recovered, placed on acidified glucose-peptone agar (pH 4.5), 10 per plate, and incubated at 25° C; germination was recorded after 3 days. Since the figures obtained for corresponding replicates were very similar, only the means are given in Table 1.

Formalin was ineffective in both covered and uncovered soil, and D-D was ineffective in uncovered soil but killed all the sclerotia when the soil was covered. Chloropicrin killed most of the sclerotia and chlorobromopropene killed all the sclerotia in both series.

A species of *Trichoderma* (probably *T. viride* Pers. ex Fr.) appeared in association with many of the sclerotia recovered from formalin-treated soil; this was also reported by Davey and Leach (1).

Different doses of fumigants.—The methods used were similar to those described above, except that only uncovered tins were used and different doses of the fumigants were applied. The temperatures of the frames varied between 19° and 30° C during the experiment, and the treatments were in duplicate.

D-D mixture and formalin were ineffective at all dosages (Table 2). Chloropicrin killed most of the sclerotia at each dosage, but the size of the dose had little effect on the extent of the kill. Chlorobromopropene killed all the sclerotia at the 2 highest dosages but was relatively ineffective at the lowest. The species of *Trichoderma* referred to above again appeared on sclerotia taken from formalin-treated soil.

TABLE 2.—*Germination of sclerotia of Sclerotium rolfsii following treatment (in soil) with various fumigants at different dosages*

Fumigant	Dose per tin (ml) [a]	Percentage germination [b]
D-D mixture	1.0	100
	1.5	100
	2.0	100
Chlorobromopropene	0.2	50
	0.6	0
	1.0	0
Chloropicrin	1.0	9
	1.5	9
	2.0	6
Formalin	1.0	100
	2.0	100
	3.0	100
Control (water)	3.0	100

[a] Dosage per 1200 g soil.
[b] Each figure is the mean of 2 tests, each with 50 sclerotia per treatment.

Fungicides as solutions or suspensions applied as soil drenches.—The sclerotia were placed in the soil in the way described above, and the soil was drenched with 500 ml of a solution or suspension of the fungicide. The treatments were in duplicate, and the tins were placed in frames in which the temperature varied between 9° and 16° C during the experiment. The sclerotia were recovered after 2 days and their viability tested.

Sodium dehydroacetate and SR 406 were ineffective even at the relatively high concentration of 0.5 per cent, and formalin killed the sclerotia only when used as a 2 per cent solution (Table 3).

CONTROL OF DISEASE BY SOIL FUNGICIDES.—*Fumigants and fungicidal solutions and suspensions.*—The mate-

TABLE 3.—*Germination of sclerotia of Sclerotium rolfsii following treatment (in soil) with various fungicidal drenches*

Fungicide	Percentage concentration [a]	Percentage germination [b]
Sodium dehydroacetate	0.5	100
	0.25	100
	0.125	100
	0.063	100
SR 406	0.5	100
	0.25	100
	0.125	100
	0.063	100
Formalin	2.0	0
	1.0	40
	0.5	45
	0.25	86
Control (water)		100

[a] Amount applied: 500 ml per 1200 g soil.
[b] Each figure is the mean of 2 tests, each with 50 sclerotia per treatment.

TABLE 4.—*Emergence of sugar beet seedlings when seed was sown in soil that had been infested with sclerotia of Sclerotium rolfsii and then treated with various fungicides as fumigants or as drenches*

Fungicide	Amount applied per tin (ml) [a]	Mean percentage final stand [b]
Chlorobromopropene	0.6	48
Chloropicrin	1.0	61
Sodium dehydroacetate	500 (0.5% suspension)	73
SR 406	500 (0.5% suspension)	73
Formalin	500 (2% solution)	72
None (inoculum added)		30
None (no inoculum added)		74
LSD (P = 0.05)		15
(P = 0.01)		27

[a] Fumigants were injected into soil; others were added to the surface as drenches.
[b] Percentage of the seed sown. Each figure is the mean of 5 replicates, each sown with 30 seeds.

TABLE 5.—*Emergence of sugar beet seedlings when seed was sown in soil that had been infested with sclerotia of Sclerotium rolfsii and then mixed with various fungicidal dusts*

Fungicide	Dosage [a] (percentage in soil)	Mean final stand [b]
TCNB	0.2	47
	0.4	54
	0.8	74
PCNB	0.2	78
	0.4	80
	0.8	83
SR 406	0.2	81
	0.4	76
	0.8	79
None (inoculum added)		23
None (no inoculum added)		87
LSD (P = 0.05)		12
(P = 0.01)		16

[a] Dusts were mixed with upper 200 g of soil.
[b] Percentage of the seed sown. Each figure is the mean of 5 replicates, each sown with 30 seeds.

rials used in the above experiments were used to treat soil that had been contaminated with mycelium of the pathogen in the way described under *Materials and Methods*. The fumigants were injected into the lower layers of the soil by means of a hydopermic syringe, and the other fungicides were added as drenches to the surface of the soil. In 1 series of controls, 500 ml of water was added to tins containing standard inocula of the pathogen; in a second series, a noninfested medium consisting of sand and maize meal was used in place of the fungal culture. Each treatment was replicated 5 times, and the tins containing the soil were placed in well-ventilated frames (temperature range 23°–28° C) for 2 weeks. Thirty sugar beet seed were then sown in each pot in 3 concentric rings containing 15, 10, and 5 seeds. The tins were placed in a frame in which the temperature was controlled (22°–27° C) and which was kept very humid to ensure satisfactory infection in the controls. The results obtained 10 days after sowing the seed are in Table 4.

Sodium dehydroacetate, SR 406, and formalin controlled disease effectively, although these materials failed to kill buried sclerotia in earlier experiments. The plants that emerged from soil treated with SR 406 were stunted and obviously had been damaged by the fungicide. A profuse growth of *Trichoderma* sp. appeared on the surface of all soil that had been treated with formalin; it seemed to have no effect on the germination and growth of the beet.

In the soil treated with chlorobromopropene, the seedlings grew abnormally; the low emergence and final stand seemed to have been caused by the phytotoxicity of this compound rather than by the action of the pathogen, since the fungus was not visible in the surface layers of the soil as it was in the infested controls.

Chloropicrin gave some control of disease and was not phytotoxic.

Fungicidal dusts.—The procedure was the same as in the preceding experiment except that certain fungicidal dusts were well mixed with the upper 200 g of infested soil before the seeds were shown. There was no postemergence killing except in the inoculated series of controls in which it was 20 per cent of the seed sown (Table 5).

TCNB controlled disease satisfactorily only at the highest dosage, but it was highly phytotoxic at this rate; seedlings in the treated soil were stunted and grew abnormally.

In contrast, PCNB and SR 406 completely controlled disease even at the lowest concentration used, and neither was phytotoxic. It is interesting to note that PCNB reduced the rate of growth of *S. rolfsii* on agar plates much more than did TCNB (4).

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FUMIGATION OF ENCYSTED GOLDEN NEMATODE EGGS AND LARVAE UNDER CONTROLLED ENVIRONMENTAL CONDITIONS [1]

Martin B. Harrison

SUMMARY

Encysted eggs and larvae of *Heterodera rostochiensis* Wr., when treated in glass fumigation chambers with D-D, chloropicrin, ethylene dibromide, or Vapam, were much more sensitive to the nematocide when the relative humidity of the atmosphere at the time of fumigation was 100 per cent than when it was 90 per cent.

When encysted eggs and larvae were stored at a relative humidity of 10 per cent for 2 weeks prior to fumigation, they were much less sensitive to the fumigants than were encysted eggs and larvae that had been conditioned at a relative humidity of 58 per cent, except when fumigation was at a relative humidity of 10 per cent. When Vapam was used, sensitivity to the fumigant increased only for those cysts stored at 58 or 98 per cent relative humidity and treated at 98 per cent relative humidity. Encysted eggs and larvae conditioned at 98 per cent relative humidity were more sensitive than those conditioned at 58 per cent only when the relative humidity at the time of fumigation was 10 per cent and the fumigant was chloropicrin, D-D, or ethylene dibromide.

Under the conditions used, the fumigants volatilized completely and were equally effective at temperatures of 10°, 20°, and 30°C.

The action of fumigants upon nematodes in soil has been investigated by many workers. Lear (4), Stark (8), Allen and Raski (1), Peters (7), and McClellan, Christie, and Horn (6) reported that soil moisture, temperature, and type influence the extent to which a material will be effective. In very few cases has it been possible to distinguish whether the above-mentioned factors were acting upon the nematode or only on the movement of the nematocide. Hagan (2) and Hannesson (3) demonstrated that soil moisture, as it affects permeability, is the greatest single factor influencing the movement of carbon disulfide in the soil in a closed system in which adsorption is not a factor.

While the experiments to be reported here were in progress, the Belgian workers van den Brande, Kips, and D'Herde (10, 11, 12) published a report on the influence in the soil of relative humidity and temperature on the fumigation control of encysted eggs and larvae of *Heterodera rostochiensis* Wr.

The purpose of the present study was to determine the effects of relative humidity and temperature on the susceptibility of the golden nematode to fumigants. The environment of encysted eggs and larvae in soil is complex; a study of the effect of any one factor requires that the work be done in artificial systems. This was the approach that was used in this paper.

METHODS AND MATERIALS.—Golden nematode (*H. rostochiensis*) cysts intermixed with soil particles were collected by the method described by Lownsbery (5). In all tests the experimental material consisted of lots of 100 cysts separated from the accompanying debris with the aid of an entomologist's aspirator and counted into Beltsville watch glasses. The fumigation chambers used were constructed from large glass desiccators, each having a capacity of 11,090 cc (Fig. 1,A). The cover of each chamber had a center tubulure that was fitted with an adapter designed to accommodate a microburette. The fumigant metered into the chamber was caught in a shallow glass dish. The opening in the adapter was closed with a solid glass plug when the burette was removed. The solution used to maintain a specified relative humidity was placed in a large petri dish in the bottom section of the chamber. The experimental cyst material was suspended over the conditioning solution on a wire-mesh rack.

Small chambers (Fig. 1,B), of essentially the same design as the large chambers, also were assembled. The covers of these chambers were not fitted with tubulation because they were used only for the storage of experimental material under controlled conditions.

Sulfuric acid solutions as given by Stevens (9) were compared with saturated salt solutions for maintaining the desired relative humidity levels in the fumigation chambers. The actual relative humidity conditions inside the chambers were checked with a hair hygrometer. The 2 methods gave similar results: larvae emerged equally well from cysts exposed to atmospheres maintained by either type of solution.

The tests involving the use of the large and small chambers were conducted in controlled-temperature cabinets. The temperature fluctuations in these cabinets were recorded with a thermograph and found to be no greater than 2°C.

The fumigants used in each of these tests were chloropicrin (trichloronitromethane), D-D (1,2-dichloropropane; 1,3-dichloropropene), EDB W-85 (1,2-dibromoethane), and Vapam (sodium *N*-methyldithiocarbamate dihydrate).

The results of each experiment and the efficacy of the fumigants were determined by an emergence test conducted as described by Lownsbery (5).

In the first series of experiments, the golden nematode cysts that were used had been stored in the laboratory but not under controlled conditions. The tests were repeated 4 times; in each replicate, duplicate lots of 100 cysts were used. The fumigation of the encysted eggs and larvae was carried out at 20°C in the large glass chambers containing sulfuric acid solutions to maintain relative humidities of 90 and 100 per cent. The control material was maintained under similar

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FIG. 1. A) Large glass fumigation chamber. Solution controlling the relative humidity was placed in a large glass petri dish below the wire screen used to support Beltsville Syracuse dishes containing the golden nematode cysts. Fumigant was metered into the chamber with a burette fitted to the adapter in the center tubulure. B) Small relative-humidity chamber in the assembled state on the left. The component parts are shown to the right of the chamber.

environmental conditions in the small chambers.

In the second part of this work, the effect of the relative humidity at which cysts are stored prior to fumigation on their susceptibility to the fumigants was investigated. Cysts were stored in the small chambers for 2-week periods at relative humidities of 10, 58, or 98 per cent. The above-mentioned storage constituted the so-called preconditioning period. Subsamples from each of the 3 preconditioning groups were then fumigated for 24 hours in the large chambers at each of the 3 relative humidities: 10, 58, and 98 per cent.

TABLE 1.—*Emergence of larvae from golden nematode cysts that were treated at different relative humidities with various fumigants*

Treatment			Average no. of larvae that emerged [b] from 100 cysts
Fumigant	Dosage [a]	Relative humidity	
	ml	%	
D-D	0.25	100	66
		90	288
	0.5	100	1
		90	28
EDB (W-85)	2.0	100	87
		90	264
	4.0	100	3
		90	67
Chloropicrin	0.005	100	45
		90	232
	0.01	100	0
		90	20
Vapam	2.0	100	27
		90	218
	4.0	100	1
		90	19
Control		100	507
		90	618

[a] Amount allowed to evaporate into a 11,090-cc chamber containing the cysts.

[b] Number that emerged following exposure to potato-root diffusate for a period of 3 weeks at 74°C. Each figure is the average of 4 tests, each with 2 lots of 100 cysts.

The effect of temperature upon the susceptibility of the nematode to the fumigants at 100 per cent relative humidity also was investigated. Duplicate lots of 100 golden nematode cysts were fumigated for 24 hours in the large chambers at 10°, 20°, or 30°C and 100 per cent relative humidity. Controls consisted of cysts maintained under these same conditions in the small chambers.

RESULTS.—The results of the first test, which was a comparison of fumigation at 90 and 100 per cent relative humidity, are given in Table 1. In the control material, the larval emergence was about equal after exposure to either environmental condition. In contrast, the relative humidity of the atmosphere in which the cysts were fumigated had a pronounced effect on action of the fumigants. In each instance, the degree of control obtained at 100 per cent was higher than at 90 per cent relative humidity.

In the second test, the relative humidity of the environment at the time of fumigation had no appreciable effect on the efficacy of 3 of the fumigants when the encysted eggs and larvae had been kept previously at a high relative humidity (Table 2). The storage of the encysted eggs and larvae at a very low relative humidity reduced the effectiveness of all the fumigants tested. The relative humidity of the storage was particularly important when Vapam was used. In fact, Vapam was

TABLE 2.—*Emergence of larvae from golden nematode cysts that had been conditioned for 2 weeks at various relative humidities and then treated with different fumigants under 3 conditions of relative humidity*

Treatment			Average no. of larvae emerged[b] from 100 cysts that had been conditioned at the indicated percentage relative humidity		
Fumigant	Dosage[a] (ml)	Relative humidity (%)	10	58	98
None		10	710	685	655
		58	683	650	582
		98	512	413	485
D-D	0.5	10	620	700	14
		58	164	3.7	2
		98	160	2.5	1.7
EDB (W-85)	4.0	10	585	615	12
		58	193	2.5	4.7
		98	145	2.5	5
Chloropicrin	0.01	10	705	640	10
		58	151	1.2	1
		98	40	2	2
Vapam	4.0	10	635	725	675
		58	422	392	310
		98	237	10	9.7

[a] Amount allowed to evaporate into a 11,090-cc chamber containing the cysts.

[b] Number that emerged following exposure to potato-root diffusate for a period of 3 weeks at 74°C. Each figure is the average of 4 tests, each with 2 lots of 100 cysts.

effective only when encysted eggs and larvae were preconditioned at 58 per cent or at 98 per cent relative humidity and fumigated at the highest relative humidity. When D-D, ethylene dibromide, or chloropicrin were used to fumigate encysted eggs and larvae that had been stored at 10 per cent relative humidity, the control obtained was only slightly higher when the fumigation chambers were maintained at 98 per cent relative humidity than when they were maintained at 58 or 10 per cent relative humidity. In contrast, these same 3 materials were quite effective when cysts preconditioned at 58 per cent relative humidity were fumigated at 58 per cent or 98 per cent relative humidity.

Within the range used in the temperature studies, temperature had no consistent effect on the efficacy of the fumigants used (Table 3). In all cases, volatilization of the chemicals was complete.

DISCUSSION.—In the field under normal moisture conditions, the relative humidity of the air spaces in the soil is rarely as low as 90 per cent, except possibly in the upper few centimeters of dry sandy soil. Only rarely, therefore, should soil moisture conditions prior to fumigation in the field have any appreciable effect on the susceptibility of encysted eggs and larvae to at least 3 of the fumigants used. Prefumigation conditions may sometimes be critical when Vapam is the fumigant, particularly if the relative humidity at the time of fumigation is low. It has been suggested that the activity of Vapam depends on its contact with water and the subsequent liberation of a toxic substance. Presumably, this moisture could be provided by the preconditioned cyst or by the atmosphere in the soil.

TABLE 3.—*Emergence of golden nematode larvae from cysts following fumigation at 100 per cent relative humidity with various fumigants at 3 different temperatures*

Treatment		No. of larvae that emerged[b] after treatment at the indicated temperature (°C)		
Fumigant	Dosage[a]	10°	20°	30°
	ml			
D-D	0.25	62	89	75
	0.5	2	5	1
EDB (W-85)	3.0	100	72	83
	4.0	5	7	15
Chloropicrin	0.005	52	65	65
	0.01	0	0	0
Vapam	2.0	72	60	53
	4.0	2	1	3
Control		353	401	389

[a] Amount allowed to evaporate into a 11,090-cc chamber containing the cysts.

[b] Number that emerged following exposure to potato-root diffusate for a period of 3 weeks at 74°C. Each figure is the average of 4 tests, each with 2 lots of 100 cysts.

If conditions are such that the requisite moisture is supplied by neither, larvae would be relatively insensitive to Vapam.

When encysted eggs and larvae are stored in the laboratory prior to *in vitro* screening tests, moisture conditions prior to fumigation may have a marked effect on their susceptibility to a fumigant. Variations in the screening results could be expected if different lots are stored under widely different moisture conditions.

The results suggest that relative humidity at the time of fumigation is much more important than is temperature. Nevertheless, soil-fumigation studies indicate that nematode control is better at high temperatures than at low temperatures (4, 8). Perhaps this effect of temperature on efficacy is the result of an effect of temperature on the rate of diffusion of the fumigant in the soil.

The fact that relative humidity at the time of fumigation had a marked effect on efficacy in these tests suggests that relative humidity may also be important in space fumigation. Although the fumigants tested are not normally used for space fumigation, it may be that the same factors may be operative with still other fumigants.

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A BRUSH-EXTRACTION METHOD FOR TRANSMISSION OF VIRUSES[1]

C. E. Yarwood

SUMMARY

When a stiff poster brush was stroked over the surface of a hairy leaf of a virus-infected donor host and then over the surface of a healthy suscept, a high level of infection usually resulted with tobacco mosaic virus, tobacco ring spot virus, cucumber mosaic virus, peach yellow bud mosaic virus, peach ring spot virus, apple mosaic virus, spotted wilt virus, and alfalfa mosaic virus. The use of Carborundum increased the efficiency of the method in all cases, and the use of phosphate increased the efficiency of the method in inoculations on bean and cowpea.

The finding of Bawden, Hamlyn, and Watson[2] that certain viruses occur predominantly in the epidermis of their hosts suggested that this tissue would be a suitable source of inoculum. A simple method of *in situ* extraction of sap from the epidermis was suggested in part by the finding of Hildebrand[3] that punctured cells exude drops of sap. A stiff poster brush, as used by Takahashi,[4] was dipped in water and stroked over the Carborundum-dusted surface of the donor host. This same brush was then immediately stroked over the Carborundum-dusted surface of the suscept. This combined operation of extraction and inoculation (not including dusting of the leaves with Carborundum and washing of the brush) required about 5 seconds. By contrast, the standard method of grinding a small bit of tissue with water in a mortar and stroking this tissue suspension over the Carborundum-dusted leaf surface required about 20 seconds, again excluding time for preparation and washing.

This brush-extraction method, with several variations, has been used successfully in the transmission of tobacco mosaic virus from tobacco to tobacco and bean, of tobacco ring spot virus from bean to bean and cucumber, of cucumber mosaic virus from cucumber to cucumber, of peach yellow bud mosaic virus from cowpea and bean to cucumber and cowpea, of peach ring spot virus from bean to cucumber, of apple mosaic virus from tobacco to bean, of tomato spotted wilt virus from tobacco to cowpea, and of alfalfa mosaic virus from tobacco to bean, cucumber, and cowpea. Since all these viruses have been transmitted by conventional mechanical methods, the only advantage claimed for the present method is speed and convenience. The only viruses tested that have failed to be transmitted by this

[1] Accepted for publication May 23, 1957.
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brush extraction method have been curly top virus from tobacco and aster yellows virus from plantain. Transmission of apple mosaic virus from tobacco to apple, of peach ring spot virus from peach to cucumber, and of peach yellow bud mosaic virus from broad bean to cowpea have been unsuccessful by the brush-extraction method, but transmission of these same viruses between other hosts has been successful, as indicated above.

The success of this brush-extraction method depends on the degree of stiffness of the brushes used and on the pressure used in stroking the leaves; neither of these can at this time be stated quantitatively. Hog-hair poster brushes (Devoe and Reynolds #812, 8; and Art and Science, Universal #713, 10) have been used. The stiffness of these brushes can be modified by changing the length of their bristles and by soaking them in water for various periods at different temperatures. As manufactured, the bristles of these brushes extend about 23 mm beyond the metal holder, but the bristles can be clipped to a shorter length to give a stiffer brush. The brushes were washed in soap and water after use.

The variations in the method that have been studied include absence or use of Carborundum on donor and suscept, absence or use of phosphate on donor and suscept, absence or use of water on donor and suscept, and use of upper and lower leaf-surfaces of the donor host. In most cases these variations have been compared with the standard method of juice inoculation described; 1 per cent tissue was used as inoculum. Local-lesion suscepts have been mainly used, so that variations in the success of different methods could be compared quantitatively. Such comparisons are of relatively little value because the dosage of inoculum in the brush-extraction method is unknown; however, it was found that the brush-extraction method usually gave more lesions than did 1 per cent tissue with tobacco mosaic virus and apple mosaic virus but fewer lesions than did 1 per cent tissue with alfalfa mosaic virus.

The greatest numbers of lesions resulted with tobacco mosaic virus. When infected tobacco leaves were stroked on their upper or lower surfaces and the brush then stroked over Pinto bean leaves that had been dusted with Carborundum and sprayed with 1 per cent K_2HPO_4, more than 100 lesions per sq. cm usually resulted. Stroking the donor host with water in the brush resulted in more subsequent lesions than did stroking with a dry brush. Addition of Carborundum or of phosphate to the donor host or to the suscept before stroking resulted in more lesions than when these were omitted. The increase in lesions as a result of adding Carborundum or phosphate or both to the suscept was greater when these same materials had not been applied to the donor than when they had. Similar results were obtained with inoculations made on a dry suscept and on one sprayed with water.

Very few lesions resulted from attempts to transmit peach yellow bud mosaic virus from cowpea to cowpea and cucumber and from attempts to transmit spotted wilt virus from cowpea to cowpea. Since the brush-extraction method of inoculation was highly successful with peach yellow bud mosaic virus when French bean was the donor host and with spotted wilt virus when tobacco was the donor host, and since juice transmissions of these viruses from all hosts indicated were successful, it seems likely that the comparative lack of success in transmission from cowpea and broad bean was due to lack of or scarcity of hairs on these donor hosts. When the donor host was stroked sufficiently hard to break the epidermis, the amount of virus obtained from nonhairy leaves was greatly increased. For example, in 3 tests on the transfer of spotted wilt virus from cowpea to cowpea, light extraction stroking of the donor host produced 41, 13, and 2 lesions per assay leaf, whereas severe extraction stroking produced 175, 70, and 39 lesions, respectively, on the opposite assay leaves.

When apple mosaic virus in tobacco (1 month after inoculation) was assayed from the inoculated leaves and from progressively higher leaves of a systemically infected and recovered plant,[5] the numbers of lesions that resulted in Pencil Pod bean from inoculum taken from the upper and lower surfaces, respectively, were as follows: inoculated leaf—0 and 0 lesions; lower leaf with "shock" symptoms—37 and 1 lesions; upper "shock" leaf—14 and 5; first recovered leaf—12 and 5; fourth leaf above—335 and 65; leaf near terminal —134 and 64; and second leaf near terminal—176 and 126. It therefore appears that the method is useful for showing differences in the amount of extractable virus in the upper and lower epidermises and at different levels of the plant. No differences were detected, however, in the amount of tobacco mosaic virus extracted from the upper and lower surfaces of tobacco or of peach ring spot virus from the upper and lower surfaces of bean.

Discussion.—Since the brush-extraction method of inoculation has been highly successful only when the donor host has hairy leaves, it is presumed that most of the inoculum comes from the hair cells. These hair cells of plants infected with tobacco mosaic virus are known to contain large amounts of virus,[6] and presumably this may be the case also with other viruses. During the brushing of the donor host, such hairs would be broken and some of the virus within them would be deposited on the brush. This freshly extracted virus would then function as inoculum when the brush was stroked over the surface of the suscept.

No claim is made that this method of inoculation is generally superior to others. The unique features of the method are believed to be that it takes the inoculum from the upper or lower epidemis at will and that it is rapid.

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[6] Nixon, H. L. 1956. An estimate of the number of tobacco mosaic virus particles in a single hair cell. Virology 2: 126-128.

RELATION OF TEMPERATURE AND HOST NUTRITION TO ANGULAR LEAF SPOT OF CUCUMBER [1]

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SUMMARY

Six isolates of *Pseudomonas lachrymans* from widely separated geographical areas were found to be similar in pathogenicity and cultural characteristics. The organism did not use inorganic salts as a source of nitrogen. It made best growth when either glutamine or asparagine was used as a source of N. When 100 μg N/ml was used, 8 mg/ml of sucrose was required for maximum growth. Above 200 μg N/ml, 1 mg/ml or less of sugar was needed. In cucumber plants grown in balanced nutrient and in soil in the greenhouse, old leaves were resistant and young leaves approaching mature size were the most susceptible. Old leaves were relatively low and young leaves were relatively high in amino nitrogen. When 3 levels of N were supplied in an otherwise balanced nutrient to cucumber plants inoculated with the pathogen, the disease index and the amino-nitrogen content of leaves increased with each increase in nitrogen level in the nutrient. At the highest nitrogen level (528 ppm), all leaves were very susceptible and the amino nitrogen content of both old and young leaves exceeded 200 μg/g of fresh tissue. In plants very deficient in potassium, the disease index was nearly as high as in high-nitrogen plants and the amino-nitrogen content, especially of old leaves, was high. When inoculated plants were exposed to 4 temperature treatments, the disease was most severe when the day temperature was 16°C and the night temperature was 28° (12-hour alternating periods). This was followed, in descending order with respect to index, by 16° continuous, 28° continuous, and 28°–16° (day-night) treatments. The highest concentration of amino nitrogen in leaves was in the 16°–28° (day-night) treatment followed in descending order by the 16° continuous, 28°–16° (day-night), and 28° continuous treatments.

The spasmodic epidemics of angular leaf spot (incited by *Pseudomonas lachrymans* (E. F. Sm. & Bryan) Carsner) of cucumber (*Cucumis sativus* L.) in Wisconsin have never been satisfactorily related to any definite sequence of climatic events. Wiles and Walker (17) pointed out that the organism grew most rapidly in nutrient broth culture at about 24°C and that disease development was greatest at 24° and 28°. They also showed that water congestion of the leaves before inoculation increased the size of the lesions and severity of the disease.

In Wisconsin, the crop grown for processing is commonly planted in rows 6 ft. apart on light sandy soil and is side-dressed with a nitrogen fertilizer about the time vines begin to run. Epidemics most commonly appear at this stage following heavy winds accompanied by rain. Since much of the soil is not covered by foliage at this time, it is believed that wind-borne infested soil particles are an important means of dissemination of the pathogen and that they create wounds that facilitate penetration. The organism has been shown to remain viable in soil during a growing season and the subsequent winter (16). In this investigation, it was observed repeatedly that leaves approaching maximum size at the time conditions were favorable for infection were severely diseased, whereas older leaves and young unfolding leaves at growing tips of stems were relatively free from lesions. As new leaves continued to develop, they remained almost or entirely free from lesions unless periods favorable for dissemination and infection recurred. Carsner (5) reported a similar situation on plants inoculated in the greenhouse. Burger (4) observed in Florida that the disease was most severe on plants supplied with a heavy application of fertilizer.

This investigation is concerned with the relation of nitrogen and carbohydrate levels to growth of the pathogen in culture medium and with the relations of constant and alternating temperatures and of host nutrition to disease development.

SOURCES AND CHARACTERIZATION OF CULTURES.—Six isolates of the pathogen were collected. Two cultures were isolated from infected cucumber leaves, 1 from Wisconsin and 1 from Colorado. Two cultures were obtained from W. H. Burkholder, Cornell University, 1 from R. G. Grogan, University of California, and 1 from S. P. Doolittle, U. S. Department of Agriculture. Single-cell isolations were derived according to the method of Lederberg (9). All isolates grew well and maintained a very uniform degree of pathogenicity when transferred frequently on beef-extract peptone agar. Gelatin stabs were liquefied slowly at 20°C; a wide infundibuliform hollow had formed by 14 days. Carbohydrate fermentation was studied by cultivation in liquid medium. Acid, but no gas, was formed in media containing 1 per cent glucose, sucrose, fructose, or xylose. Lactose and maltose were not fermented. Starch was not hydrolysed. Hydrogen sulfide production, nitrate reduction, and indole formation did not occur. A close agreement of all isolates indicated a high degree of uniformity in this organism despite their diverse origins.

METHODS OF INOCULATION AND DETERMINATION OF DISEASE INDEX.—The Ohio MR25 variety of cucumber was used in all inoculation experiments. To avoid the effect of prolonged periods of poor illumination in a

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a moist chamber, inoculations were made *in situ* in the greenhouse without the use of a high-humidity chamber. Abundant infection occurred when the bacterial suspension was sprayed on the under surfaces of the leaves from an atomizer attached to an airline at a pressure of 20 lb./sq. in. When this method was used, minute water-soaked lesions appeared on young leaves in about 3 days; on old leaves, considerably fewer lesions appeared 1 to 2 days later.

Unless otherwise indicated, disease readings were made on leaves that had just reached maximum size or were approaching that stage at the time of inoculation. Such leaves are referred to as "young leaves." Leaves that had reached maximum size 2 or more days before the time of inoculation are referred to as "old leaves." Plants were examined every day for 7 days after inoculation, and final readings were made at 14 days. Each plant in a given treatment was placed in 1 of the following disease classes: 0 = no visible lesions; 25 = less than 5 lesions per leaf; 50 = 5–50 lesions; 75 = more than 50 but less than 100 lesions; 100 = 100 or more lesions. A disease index for each treatment was calculated by multiplying the number of plants in each class by the class; number, summing the products, and dividing by the total number of plants.

METHODS OF TISSUE ANALYSIS.—Assays for sugar and amino nitrogen were made with leaves from non-inoculated plants grown at various temperatures and nutrient levels in order to determine whether or not the levels of these materials were correlated with disease development in comparable inoculated plants. Fresh leaf samples were collected in Petri-dish moist chambers to prevent loss in fresh weight. The midrib of the leaf was removed, and a 1-g sample of the remaining tissue was weighed. The second or third true leaf from the base of the plant was used for the old-leaf sample, and the last leaf to reach mature size and the next youngest leaf were used for the young-leaf sample. The 1-g sample was ground with a mortar and pestle, and the tissue was mixed with 5 ml of distilled water. The mixture was filtered through 1 layer of cheesecloth. One ml of the filtrate was diluted with 3 ml of distilled water and placed in a 16 × 125-mm test tube. Four ml of saturated lead acetate solution was added, and the mixture was centrifuged at 5000 rpm for 10 minutes. Eight ml of saturated dibasic sodium phosphate was then added and the mixture centrifuged at 5000 rpm for 10 minutes. The supernatant was poured immediately into a clean test tube.

Soluble carbohydrates were determined according to the method of Seifter et al (13). Two ml of distilled water was added to 1 ml of the supernatant in a test tube, which was then placed in a cold-water bath. Each tube received 6 ml of anthrone reagent and was returned to the cold bath at once. After all tubes were so treated, they were placed in boiling water for 10 minutes, then cooled and read immediately on a Beckman spectrophotometer at 620 mμ. A standard graph was prepared by running a set of sucrose standards through the same procedure. All readings were converted from the standard graph.

A 2-ml sample of the original supernatant was assayed for amino nitrogen according to the procedure outlined by Smith and Agiza (14). The standard consisted of 3 amino acids: arginine, aspartic acid, and glutamic acid in the ratio of 3:1:1. Bonner (3, p. 293-318) lists these 3 acids as occurring most frequently at the ratio indicated in the leaves of numerous plants. The set of standards gave a straight-line graph against concentration up to 60 μg per ml. Above this, the readings on the spectrophotometer decreased slightly but this was of little importance in this case because few samples of the supernatant ever reached that concentration.

GROWTH OF PSEUDOMONAS LACHRYMANS IN RELATION TO CARBOHYDRATE AND NITROGEN CONTENT OF THE CULTURE MEDIUM.—When it became evident that the nitrogen balance in the host had an important bearing on disease development, a correlative study was made of the growth of the pathogen in culture in relation to carbohydrate and nitrogen content of the medium. All glassware was acid-washed, then rinsed in 0.1 N HCl and finally in distilled water. The basal synthetic medium consisted of 0.3 g KH_2PO_4, 1.2 g K_2HPO_4, and 0.3 g $MgSO_4 \cdot 7H_2O$ per liter of distilled water and 1 ppm each of Fe, Cu, Zn, and Mn. Sucrose was used as the carbohydrate source in all experiments. The nitrogen compounds were supplied as indicated later. The medium was buffered at pH 6.9. Fifty-ml portions of the medium in 125-ml Erlenmeyer flasks were autoclaved at 15 lb. pressure for 15 minutes. Single-cell cultures of the organism were grown on beef-broth peptone agar slants for 12 hours and removed by washing each slant with 1 ml of sterile distilled water. The bacterial suspension was diluted to an optical density of between 0.03 and 0.02 as measured on a Lumetron spectrophotometer at 490 mμ. A Cornwell pipetting syringe was used to seed each flask with 1 ml of the suspension. The seeded flasks were incubated on a continuous shaker for a period of 36 hours. This time period was chosen to avoid large errors due to variable lag phases and to large numbers of dead cells. Amount of growth was determined by measuring optical density.

No growth occurred on culture media containing 0.2 per cent sucrose and inorganic nitrogen (100 μg/ml) supplied as NH_4Cl, $(NH_4)_2SO_4$, NH_4NO_3, or $NaNO_3$, with and without the addition of a mixture of 7 water-soluble vitamins. Of 15 amino acids tested as sources of nitrogen, only glutamine, asparagine, arginine, and aspartic acid supported growth. Glutamine and asparagine supported the best growth, whereas pigment production was best with asparagine.

When various amounts of sucrose and asparagine were used, it was found that the critical nitrogen level for growth was 100–200 μg N/ml of medium. With 100 μg N/ml, 8 mg of sucrose per ml was required to produce maximum growth of the organism. Table 1 gives the average optical densities of 3 replicates of 1 isolate. Similar results were obtained with 4 other isolates. Best pigment production occurred in medium with a high level of nitrogen and a low level of sucrose.

TABLE 1.—*Growth (as indicated by optical-density readings) of Pseudomonas lachrymans on media containing various concentrations of sucrose and asparagine*

Amount of sucrose (mg/ml)	Optical densities when amount of N (μg/ml) was as indicated [a]				
	50	100	200	400	800
0	0.2	0.5	1.4	2.5	4.5
1	0.8	1.2	4.5	4.8	5.1
2	1.8	2.3	4.8	5.1	5.3
4	2.3	2.8	5.1	5.2	5.3
8	2.5	4.7	5.2	5.3	5.5

[a] Each value is the average of density readings of 3 replicate flasks. Readings were made after incubation for 36 hours on a continuous shaker. Optical density was measured with a Lumetron spectrophotometer at 490 mμ.

RELATION OF HOST NUTRITION TO DISEASE DEVELOPMENT.—The continuous-drip equipment and quartz-sand culture technique described by Gallegly and Walker (7) were used. The basal Hoagland's nutrient solution contained 213 ppm nitrogen, 31 ppm phosphorus, and 234 ppm potassium. This was modified to provide solutions in which N, P, and K were adjusted to higher or lower levels than those in the basal solution. Seven-day-old seedlings grown in vermiculite were selected for uniformity of size and transplanted into drip-system pots. They were inoculated 1 month later, and disease readings were made 14 days after inoculation.

The widest range in disease indices was found in the series in which nitrogen was varied from 1.5 to 528 ppm with phosphorus and potassium levels held constant (Table 2). Plants grown at the lowest level of nitrogen were stunted and chlorotic. The few lesions that did develop appeared in 5–6 days. They remained small and were confined to the 2 youngest leaves. On plants grown in basal solution (213 ppm N), lesions were visible in 3 days and increased in size up to approximately 2 mm in diameter; they had chlorotic borders. The young leaves had the greatest number of lesions. Plants grown at 528 ppm nitrogen were dark green in color. The lesions appeared in 2 days and increased in diameter up to 3 mm. All leaves were about equally susceptible, and some lesions appeared on stems and petioles.

In the series in which potassium was varied but nitrogen and phosphorus were not, the only indication of effect on disease development was in the nutrient in which the potassium content was 1 ppm. Lesions appeared in 2 days and enlarged rapidly. Water-soaking of the leaves and stems was evident. The effect of low potassium is in accord with the results of Johnson (8), who reported that potassium-deficient tobacco plants were subject to water congestion and were more susceptible to wildfire (incited by *Pseudomonas tabaci* (Wolf & Foster) F. L. Stevens) and blackfire (incited by *P. angulata* (Fromme & Murray) Holland). Thaung and Walker (15) found that high nitrogen, low potassium, and low phosphorus predisposed lima bean plants to bacterial blight (incited by *P. syringae* v. Hall). They also found that low nitrogen decreased the susceptibility of lima bean plants. Bachelder et al (2) found that excessive nitrogen increased susceptibilty of peach fruit to bacterial spot (incited by *Xanthomonas pruni* (E. F. Sm.) Dows.).

In the present investigation, variation in phosphorus had no effect on disease expression.

Noninoculated plants grown in nutrient solutions with some of the same levels of nitrogen and potassium just described were assayed for soluble carbohydrates and amino nitrogen. The data in Table 3 are averages from assays made on leaves taken at 2 p.m. from 3 plants in each of 2 experiments. In the series in which

TABLE 2.—*Development of angular leaf spot on cucumbers grown (for 1 month prior, and 14 days after, inoculation) in nutrient solutions with various levels of nitrogen, phosphorus, and potassium*

Amounts of N, P, and K (ppm) in nutrient			Disease indices [a] in 5 experiments					
N	P	K	1	2	3	4	5	Mean
1.5	31	234	25	25	12	25	37	25
7.5	31	234	50	37	37	50	50	45
213	31	234	62	50	62	50	62	57
528	31	234	75	75	87	75	87	80
213	1	234	37	65	50	62	62	55
213	31	234	62	50	62	50	62	57
213	80	234	50	50	62	50	62	55
213	31	1	75	62	75	62	62	67
213	31	6.5	50	62	50	50	62	55
213	31	234	62	50	62	50	62	57
213	31	1156	62	50	50	37	50	50
LSD (5 per cent)								8
LSD (1 per cent)								13

[a] Nine plants were used for each treatment and experiment. An index of 0 indicates no disease; an index of 100 indicates that all plants had 100 lesions or more on each leaf that just reached maximum size or nearly so at the time of inoculation.

TABLE 3.—*Soluble carbohydrate and amino nitrogen (fresh-weight basis) in leaves of cucumber grown in various nutrient solutions* [a]

Amount of N, P, and K (ppm) in nutrient			Age of leaves assayed	Carbohydrate (mg/g)	Amino nitrogen (μg/g)
N	P	K			
1.5	31	234	Young	4.7	92
			Old	3.6	64
213	31	234	Young	5.1	371
			Old	3.3	171
528	31	234	Young	6.8	485
			Old	3.9	200
213	31	1	Young	5.9	150
			Old	4.7	235
213	31	6.5	Young	5.2	100 [b]
			Old	3.3	150
213	31	65	Young	5.0	185 [b]
			Old	2.5	114

[a] Each figure is the average of values from 3 plants in each of 2 experiments except where indicated otherwise. The leaves were collected for assay at 2 p.m.

[b] Figures are averages of values from 3 plants in 1 experiment.

nitrogen was varied, the amino nitrogen was consistently higher in the young than in the old leaves. As nitrogen in the nutrient increased, amino nitrogen increased in both young and old leaves. Thus, there was a direct correlation between amino nitrogen and disease index (Table 2). When potassium was at a low level, there was more amino nitrogen in old leaves than in young leaves. This was also correlated with a higher disease index in potassium-deficient plants.

Arthur et al (1) and Dearborn (6) reported that the percentage of soluble nitrogen in leaves of cucumber plants grown in solutions high in nitrogen was 2–3 times greater than that in plants grown in a balanced solution. Reid (11) found the same to be true for squash seedlings and reported further (12) that the percentage of nitrogen in fresh tissue of 28-day-old plants was highest in leaves approaching maturity and lowest in the youngest leaves and in the old leaves. Nightingale et al (10) found that the percentages of amino and amide nitrogen were higher in the lower leaves of tomato plants grown in a solution low in potassium than they were in the upper leaves of the same plant or in the upper and lower leaves of plants grown in a solution high in potassium.

In the present tests, soluble carbohydrates were always higher in young leaves than in old leaves from plants receiving the same treatment. There was no consistent correlation between carbohydrate content and disease index.

RELATION OF CONSTANT AND ALTERNATING AIR TEMPERATURES TO DISEASE DEVELOPMENT.—Plants were grown singly in soil in 4-in. clay pots at 25° ± 2° C until they had reached the 5- to 6-leaf stage (approximately 4 weeks). They were selected for uniformity of size, and 4 plants were placed in each of the following temperature treatments: 28° continuous; 16° continuous; 28° day, 16° night (12-hour schedule); and 16° day, 28° night (12-hour schedule). After exposure for 4 days to the respective temperature treatments, plants were inoculated and continued in the same treatments. Disease readings were made 14 days after inoculation. Five similar experiments were performed during winter months in the period from December 15 to March 25.

TABLE 4.—*Development of angular leaf spot in cucumber plants grown at 4 different temperatures*

Temp. treatment [a]	Disease indices [b] in 5 experiments					
°C	1	2	3	4	5	Average
16° Continuous	62	81	75	75	68	72
16° Day, 28° night	87	100	100	90	90	93
28° Day, 16° night	50	56	45	45	50	49
28° Continuous	62	50	59	62	56	58
LSD (5 per cent)						9
LSD (1 per cent)						14

[a] Plants were predisposed to the temperature treatments 4 days prior to inoculation and continued in the same treatments for 14 days after inoculation.

[b] Four plants were used in each treatment and experiment. An index of 0 indicates no disease; an index of 100 indicates that all plants had 100 lesions or more on each leaf that had just reached maximum size or nearly so at the time of inoculation.

Lesions appeared in 2 days on plants in the 16°–28° (day-night) treatment, and old and young leaves were about equally affected. On the plants in the 28°–16° (day-night) and 28° continuous treatments, lesions appeared in 3 days and were confined almost entirely to the young leaves. In the 16° continuous treatment, lesions appeared in 6–7 days and old and young leaves were about equally affected.

In 4 of the 5 experiments, the lowest disease index was in the 28°–16° (day-night) treatment (Table 4). With the exception of 1 experiment, the disease indices of the 28° continuous and 16° continuous treatments were intermediate between those of the alternating-temperature treatments. The data were treated statistically by analysis of variance. The differences among the mean disease indices of the 16°–28° (day-night), 16° continuous, and 28°–16° (day-night) treatments were significant at the 1 per cent level. The difference between the index for the 28° continuous treatment and that of the 28°–16° (day-night) treatment was barely significant at the 5 per cent level. The fact that the disease was more severe at the 16° continuous than at the 28° continuous treatment is at variance with the results of Wiles and Walker (17), who kept plants in moist chambers for 3 days after inoculation; in the present investigation, plants were inoculated *in situ* without exposure to high humidity.

Young and old leaves from plants exposed to the same temperature treatments were assayed for soluble carbohydrates and amino nitrogen at 4 intervals over a 12-hour daylight period (Table 5). The amino-nitrogen content of the leaves was lowest in the morning, reaching a maximum at 2 p.m. in some treatments and at 6:30 p.m. in others. The plants in the 16°–28° (day-night) treatment had the highest average concentration of amino nitrogen, followed in descending order by those at 16° continuous, 28°–16° (day-night), and 28° continuous treatments. When the average amino-nitrogen levels of young leaves of the 16°–28° (day-night), 16° continuous, and 28°–16° (day-night) treatments are compared with disease indices of inoculated plants (Table 4), it is evident that there was a positive correlation between disease index and amino-nitrogen level. There was no correlation between amino nitrogen and disease when 28° continuous and 28°–16° (day-night) temperatures were compared. The old leaves were consistently lower in amino nitrogen than were the young leaves of the same plants. Moreover, the lesions on old inoculated leaves were relatively few in number except in the 16°–28° (day-night) treatment where they were nearly as numerous on old as on young leaves. The old leaves in this treatment had the highest average amino-nitrogen content of such leaves in the 4 treatments.

In general the soluble carbohydrate level was lower in the old leaves than in young leaves, and there were some differences between the levels in the 4 temperature treatments. At any time of the day, however, the

TABLE 5.—*Soluble carbohydrate and amino-nitrogen contents (on a fresh-weight basis) of leaves of cucumber plants exposed to 4 temperature treatments for 4 days*[a]

Temperature treatment °C	Age of leaf assayed	Carbohydrate (C) and amino nitrogen (N) at times of the day indicated									
		6:30 a.m.		10 a.m.		2 p.m.		6:30 p.m.		Average	
		C	N	C	N	C	N	C	N	C	N
		mg/g	μg/g	mg/g	μg/g	mg/g	μg/g	mg/g	μg/g	mg/g	μg/g
16° Continuous	Young	7.0	171	10.0	228	7.5	285	9.1	400	8.4	271
	Old	4.1	85	5.0	114	5.4	214	4.1	142	6.6	139
16° Day, 28° night	Young	3.7	185	5.0	214	6.2	428	7.9	571	5.7	349
	Old	3.3	85	5.0	114	5.4	214	5.0	171	4.7	146
28° day, 16° night	Young	6.5	100	7.5	85	6.2	142	6.2	200	6.6	132
	Old	2.9	57	5.4	28	5.0	100	3.7	114	4.2	75
28° Continuous	Young	5.0	85	12.5	128	5.8	142	3.7	142	6.7	124
	Old	4.5	57	5.8	57	5.8	100	3.7	100	4.9	78

[a] Values are averages of 2 experiments. Two plants were used in each treatment and experiment.

carbohydrate was at a level sufficient to promote good growth of the organism as evidenced by the data obtained in studies of the organism on culture medium. It appeared, therefore, that amino nitrogen was more important than was carbohydrate in affecting growth of the pathogen after penetration and the severity of disease symptoms.

DISCUSSION.—It was observed repeatedly in the field and greenhouse that old leaves of cucumber plants were highly resistant, whereas leaves approaching maximum size were very susceptible to infection by the angular-leaf-spot pathogen. Exceptions to this were noted when plants were grown in nutrient high in nitrogen or low in potassium. In general, old leaves were low in amino nitrogen; in these exceptional cases, however, the amino nitrogen was higher than normal in the old leaves. Old leaves also were susceptible when plants were subjected to certain temperature treatments; here again, treatments that made old leaves susceptible also resulted in amino-nitrogen contents higher than normal. Since the organism requires organic nitrogen in culture medium for growth and since there was a consistent correlation between amino-nitrogen content of leaves and severity of disease, it is believed that amino-acid content of leaf tissue is a primary factor in determining the susceptibility of cucumber leaves to infection. Although the soluble carbohydrate content of old leaves was generally lower than that of young leaves, it was considered that even in old leaves there was sufficient carbohydrate available for the pathogen, provided adequate amino nitrogen was present. It is suggested, therefore, that soluble carbohydrate content of leaf tissue in leaves of different ages and on plants submitted to various nutrient and temperature treatments is of secondary importance in determining susceptibility.

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HOST-PARASITE RELATIONSHIP OF SPHACELOTHECA SORGHI ON SORGHUM [1]

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SUMMARY

In studies made in Egypt, susceptibility of sorghum to infection by *Sphacelotheca sorghi* was found to be limited to the period between sowing of the seed and emergence of the seedling. The length of this period differed according to the date of sowing. It was longest in early sowings (March and April) and became progressively shorter in successive later sowings. In early sowings, the amount of infection reached a maximum when chlamydospore suspension at the rate of 0.5 g per liter of water was added in equal amounts to the seedlings on the third or fourth day after sowing, whereas in late sowings maximum infection occurred when the seeds were inoculated on the same day of sowing. Incidence of infection was strongly affected by the date of sowing. It was high when seeds were sown from March to May, whereas little or no infection occurred in treatments sown during June or later. This might be due to the effect of high temperature prevailing during late sowings.

The vigor of the host plant was found to be affected by infection. Smutted plants were considerably shorter and thinner than the healthy ones.

Apparently healthy sorghum plants that had been previously inoculated proved to be free from the pathogen, since they always produced healthy secondary heads. Smutted secondary heads were produced when infected primary heads were removed by cutting the stalk at various points. This verifies previous reports that the nature of infection of the disease is systemic and that viable mycelium of the pathogen is present in all or most tissues of the host. Healthy heads were produced among smutted ones on the same plant, and healthy grains were produced in some infected heads. This suggests that the shoot apex may outgrow the pathogen.

Sphacelotheca sorghi (Lk.) Clint., which causes grain smut of sorghum (*Sorghum vulgare* Pers.), is considered to be the commonest pathogen on sorghum in Egypt and in most countries where the crop is cultivated. The disease is prevalent in upper Egypt where grain sorghum is widely cultivated as a summer crop.

This investigation was carried out to determine the period during which infection can take place on the germinating seed of the host plant. The effect of the parasite on the plant and the location of the parasitic mycelium in the host tissues were studied also.

MATERIALS AND METHODS.—Seed of 2 sorghum varieties, Giza 10 and Giza 20, was obtained from the Plant Breeding Section, Ministry of Agriculture. Seeds were disinfested by soaking them in 0.5 per cent solution of commercial formalin for 1 hour. Floating light seeds and spore masses, if present, were skimmed off. The treated seed then was spread on clean sterilized sacking and allowed to dry.

Inoculum was obtained from smutted heads collected from different locations. Chlamydospores from the smut sacs were taken out, mixed together thoroughly, and then kept in dry containers until used as inoculum.

The following 2 methods were used in the experimental procedures for planting and inoculation.

Field method.—Small holes, 5 cm in diameter and 25 cm apart, were prepared in small plots (2 × 2 m). The soil in those plots was unsterilized. Five disinfested seeds were placed in each hole and covered with a wet filter paper or cotton wool. A small pot was placed over the hole in order that the seed could be subjected to as normal a condition as possible for germination. Inoculum was applied to the seeds or later to the seedlings, 1 treatment a day from the day of sowing until the twelfth day after. At the time of inoculation, pots and filter papers were removed and a chlamydospore suspension added at the rate of 0.5 g per liter of sterilized water. The seeds then were covered gently with soil from the same plot. Ten ml of the inoculum suspension was added to each treatment.

Pot method.—Disinfested seeds were sown normally in small pots, 5 seeds in each. The soil used was unsterilized. Inoculum was applied to each, 1 treatment a day, starting from the day of sowing. Inoculated seeds or seedlings were transplanted, immediately after inoculation, to the field. The plants were spaced 25 cm apart in 2 × 2-m plots.

Controls in both methods were treated similarly except for the absence of inoculum.

PERIOD WHEN INFECTION CAN OCCUR.—It has been known that infection of sorghum by *Spacelotheca sorghi* can take place only when the host is in the seedling stage (2, 4, 5). This is accomplished by the infection hyphae that arise from the germinating chlamydospores or sporidia. The infection hyphae penetrate the host tissues through the mesocotyl while it is still under ground.

An experiment was carried out, therefore, to determine the period within which infection can take place. Sowing was on May 28, and both field and pot methods were used in planting. At the end of the growing season, results were calculated in terms of percentage of infected plants.

Infection took place only during a period limited to that between the time of germination of the seed and the time of emergence of the seedling (Table 1). This

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TABLE 1.—*Percentage infection of Giza 10 and Giza 20 grain sorghum varieties inoculated at various times and by different methods with Sphacelotheca sorghi*

Time of inoculation	Percentage of infected plants when 2 procedures were used for inoculation and sowing	
	Field method	Pot method
At sowing	56	19
After 1 day	60	28
After 2 days	25	16
After 3 days	33	6
After 4 days	10	0[a]
After 5 to 12 days, inclusive	0[b]	0

[a] Time of emergence of seedlings.
[b] Seedling emergence was after 7 days.

was concluded on the basis that infection occurred only when inoculum was applied at time of sowing or any time prior to seedling emergence. The 2 sorghum varieties used behaved similarly with respect to their disease reaction in each treatment.

The incidence of infection varied considerably with the time of inoculum application. The highest percentage of infection resulted from the application of inoculum 1 day after sowing, at which time the seed had started germination. The amount of the disease was considerably less or no infection took place when inoculum was applied 4 days or more after sowing.

The method of planting had a pronounced effect on disease development. The field method of planting seemed to create conditions that increased the amount of disease and lengthened the period during which infection could occur.

In further experiments on the effect of date of sowing on infection, disinfected seeds were planted by the field method at intervals from early March to August. In each case, an equal amount of inoculum was used for each treatment: 10 ml of a chlamydospore suspension at the rate of 0.5 g per liter. Inoculum was applied to seeds at time of planting or to seedlings at various stages of development until they were 12 days old.

The length of the period when infection can occur differed according to the date of sowing (Table 2). It was long in the case of early sowings and progressively shorter with the successive later sowings. It is obvious that in early sowings, i.e. from March up to the middle of May, percentage of infection reached a maximum when inoculum was added to the seedlings 3 or 4 days after sowing. In sowings made in June and July, however, the maximum percentage of infection occurred when seed was inoculated on the day of sowing. This can be explained on the basis that both seed germination and growth of the seedling are usually slow in early sowings, since relatively low temperatures prevail during that time. In the case of the very late sowings, i.e. in August, no infection resulted.

In the Egyptian environment, top soil within the depth of 5 cm usually reaches and maintains its maximum temperature during July and August, as has been reported by Migahid et al (6). The decrease in incidence of smut in treatments sown during July and August can be attributed to the high soil temperature prevailing on such dates.

Therefore, the foregoing results lead to the conclusion that in the case of sorghum infection by *S. sorghi*, the probability of infection decreases with the increase in soil temperature prevailing during the first week after sowing. This agrees to a great extent with the findings of Melchers and Hansing (5), who reported that temperature was a limiting factor for the development of grain sorghum smut. They found that the incidence of the disease was relatively low at high temperatures, whereas relatively low temperature was favorable for its development.

EFFECT OF THE PARASITE ON THE HOST.—Preliminary field observations showed that diseased plants were usually shorter and thinner than the healthy ones. In further tests, measurements were made on the length and circumference of many healthy and diseased mature plants grown in the same field and sown at the same time.

Plant vigor was found to be reduced considerably by the disease (Table 3). Smutted mature plants were

TABLE 2.—*Percentage infection of Giza 10 and Giza 20 grain sorghum plants[a] planted on different dates and inoculated with Sphacelotheca sorghi at various times*

Time of inoculation	Percentage of infected plants when planting date was as indicated									
	March 31	April 23	May 15	May 29	June 12	June 25	July 2	July 13	July 21	Aug. 10
At sowing	58	63	61	62	36	23	8	4	4	0
After 1 day	67	73	75	52	17	10	4	3	0	0
After 2 days	76	75	85	37	4	5	3	2	0	0
After 3 days	80	88	33	23	4	3	0	0	0[b]	0[b]
After 4 days	63	54	10	17	5	0[b]	0[b]	0[b]	0	0
After 5 days	45	52	0	0[b]	0[b]	0	0	0	0	0
After 6 days	30	19	0[b]	0	0	0	0	0	0	0
After 7 days	3	0[b]	0	0	0	0	0	0	0	0
After 8–12 days inclusive	0[b]	0	0	0	0	0	0	0	0	0

[a] Disease reaction of the 2 sorghum varieties was almost identical.
[b] Time of emergence of seedling.

TABLE 3.—*Length and circumference of healthy and smutted mature sorghum plants (Giza 20) planted in the field on various dates*

Time of planting	Plant length [a]		Stem circumference [a]	
	Healthy	Smutted	Healthy	Smutted
	cm	cm	cm	cm
March 31	192	168	6.9	5.6
April 23	199	185	5.9	5.6
May 12	225	196	5.7	4.5
May 29	217	182	6.0	5.1
June 12	252	218	5.6	5.0
June 25	230	169	5.7	4.3
July 6	177	151	4.9	4.3
July 20	144	124	4.1	3.7

[a] Each figure is an average of the measurements of 100 mature plants.

shorter and thinner than healthy ones. No other abnormal malformation was observed on the vegetative parts of the large number of diseased plants that were examined. These results, however, do not agree with those obtained by Reed in a very early paper (7). He reported that *S. sorghi* did not affect height of sorghum plants whereas *S. cruenta* made plants 6–12 in shorter than normal ones.

DEVELOPMENT OF THE PARASITE IN HOST TISSUES.—The artificially inoculated seeds, instead of producing completely smutted heads, gave one of the following variations: 1) normal plants bearing healthy heads; 2) partially smutted plants bearing both healthy and smutted heads; and 3) partially infected heads, with smut sacs occupying the top, the bottom, or the side of the head.

This phenomenon had been observed by other workers, but no definite explanation has yet been given. Martin and Ratliffe (3), working on loose kernel smut of sorghum, suggested that this phenomenon might be due to an escape from infection, whereas Melchers (4) and Davis (1) attributed it to the failure of the disease to develop despite the presence of the parasite in latent buds of the host.

An attempt was made to investigate the phenomenon in a large number of healthy plants that were produced from heavily inoculated seeds. When the plants were near maturity, stalks were cut at different levels: 1) just below the primary heads; 2) at the middle of the stalk; and 3) just above soil level. The remaining parts of the plants were left to produce secondary heads. Symptoms on the primary and on the secondary heads of the same plant were recorded.

It was found that all apparently healthy plants gave healthy secondary heads. In most cases, smutted plants gave diseased secondary heads, but in a very few cases they gave healthy secondary heads. Partially diseased plants with healthy and smutted primary heads often gave nothing but smutted secondary heads. Furthermore, the position of smut in the secondary heads was not always identical with that on the primary heads of the same plant.

Infected mature plants generally produced smutted secondary heads, irrespective of how far the stalk had been cut back (Table 4). This could be explained on the basis that infection is systemic and that mycelium of the pathogen could remain viable in buds of the host. When axillary shoots or sucker buds of the infected plant are induced to grow, the pathogen also is stimulated and invades the developing shoots causing smutted secondary heads.

TABLE 4.—*Range of symptoms [a] on secondary heads that developed in diseased sorghum plants whose primary heads had been removed [b]*

Symptoms on primary heads	Symptoms on secondary heads	Symptoms on primary heads	Symptoms on secondary heads
S	S	Pt	S, S
S	S, S	Pt	S, S, Pt
S	S, S, S	Pt	S, S, Pb, S, S, S
S	S, S, Pt	Pt	S, Pt, Ps, S
S	S, S, Ps, Pt	Pt	S, Pt, Pt, H
S	S, Pt	Pt	S, Pt, H, Pt, S
S	S, Pt, Pt	Pt	S, H
S	S, Pt, Ps	Pt	Pt
S	S, Pt, Ps	Pt	Pt, S
S	S, Ps	Pt	Pt, S
S	S, Ps, Ps	Pt	Pt, S, S, Ps
S	S, Pb	Pt	Pt, S, S, S, Ps, H
S	S, Pb, Pt	Pt	Pt, S, Pt, S
S	S, Pt, H, Pt, Pb	Pt	Pt, S, Pt, Ps
S	S, H	Pt	Pt, S, Pt, Pt, H, Pt
S	H	Pt	Pt, Pt, S, Pt
S, S	S, S, Pt	Pt	H
S, Pt	S, Pt, Pt	Pt, S	Pt, S, Pt, Ps
S, Pt	S, Pb, Ps, Ps, S, S, S	Pt, Pt	Pt, Pt
S, Pb	S, H, Pb	Pt, Pt, Pt	S, Pt, H, H, Ps
S, H	S, H	Pt, Pt, Pt Ps, Ps	S, Ps, S, S, S, S, S

[a] Abbreviations separated by commas refer to symptoms on heads recorded in succession, starting from the tip of the plant. The abbreviations indicate the following: H, healthy; S, completely smutted; Pt, partially smutted head with smut sacs at the tip; Ps, partially smutted head with smut sacs at the side; and Pb, partially smutted head with smut sacs at the bottom.

[b] Stalks were cut just below the head, at the middle of the stalk, or at ground level. This had no effect on symptoms; therefore, the location of the cut is not listed.

Frequently, healthy heads were found among smutted ones on the same plant, and also healthy grains and smutted sacs were found on the same head. This appears contradictory to the systemic nature of the infection, but it could be explained on the basis that the apical portion of the meristematic region of the stem may outgrow the mycelium of the pathogen and thus heads with healthy portions or even smut-free heads are produced occasionally.

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BIOLOGICAL STUDIES OF THE SOYBEAN CYST NEMATODE[1]

C. B. Skotland[2]

SUMMARY

Forty-five plant species representing 28 genera and 9 families were inoculated with larvae of the soybean cyst nematode, *Heterodera glycines*, in greenhouse tests. Hosts included only species of *Leguminosae*. Previously unreported hosts were *Glycine gracilis*, *Vicia villosa*, *Lespedeza stipulacea*, *L. striata*, and *L. cuneata*. Hosts reported by Ichinohe and confirmed in these tests included *Glycine ussurinesis*, *Phaseolus vulgaris*, and *P. angularis*. Mature male nematodes were observed 14 days and second-generation larvae 21 days after inoculation of soybean plants with second-stage larvae. These data suggest that 4 or 5 generations of this nematode could develop in North Carolina within a growing season. Viability of eggs and of larvae in cysts of the soybean nematode was drastically reduced by desiccation. Few larvae were recovered from cyst material placed in a Syracuse watch glass for 1 month or more and allowed to dry at room temperature. No cysts with viable contents were collected from bulb scales or from soil of narcissus bulbs that had been grown in heavily infested fields then cured and air dried for 60 days.

In 1914, a disease of soybean (*Glycine max* (L.) Merr.) incited by a nematode of the genus *Heterodera* was reported from Japan (7). It was later reported from additional areas in Japan (11, 15), Korea, and Manchuria (10). The first report of its occurrence outside the Orient was in 1955 (16). Previously this nematode had been referred to as *H. schachtii* Schmidt 1871 (1) or as *H. göttingiana* Liebscher 1892 (5). Since the host range reported for this nematode by Fujita and Muira (3) differed from that described for *H. göttingiana*, Franklin (2) concluded that it was not *H. göttingiana*. Ichinohe (8) named this nematode *H. glycines* in 1952 and later reported on its parasitism, morphology, and ecology (9, 10). Hirschmann (6) has reported distinct morphological differences between the second-stage larvae of the clover cyst nematode *H. trifolii* Goffart 1932 and those of *H. glycines*.

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Results of biological studies of *H. glycines* are reported herein. A preliminary report has been presented (13).

Materials and methods.—Cysts of *Heterodera glycines* were collected from infested fields and used to infest soil that had been fumigated with methyl bromide. Seeds of the soybean variety Roanoke were planted in 6-in. clay pots, and the plants that emerged were allowed to grow for 8 weeks. At this time, soil from these pots was screened through 20-, 60-, and 325-mesh sieves. Large numbers of cysts containing eggs as well as egg masses were collected on the 60-mesh sieve. Males were recovered on the 325-mesh sieve. In preliminary tests, the material collected on the 60-mesh sieve was placed in Baermann funnels containing distilled water and soybean root leachates. The leachates consisted of water that had percolated through potted soil in which soybean plants were growing. Half of these samples of each category were aerated by bubbling air through the solutions containing the samples. The numbers of larvae recovered when root leachates were used or when funnels were aerated were no greater than the numbers of larvae recovered in nonaerated funnels containing distilled water. In subsequent tests, larvae were recovered from nonaerated distilled water in Baermann funnels. Maximum larval emergence occurred within 10 days.

Roots were prepared for microscopic examination by fixing in osmic acid, dehydration in ethyl alcohol, and clearing in methyl salicylate.

HOST RANGE.—Plant species tested were grown in 2- and 4-in. clay pots containing soil that had been fumigated with methyl bromide. The plants were inoculated when 2–6 weeks old. A pencil was used to make a hole 1–3 in. deep alongside each plant. From 300 to 400 second-stage larvae, in water, were placed in each hole, which was closed after inoculation. Roots of plants in half of the pots were examined for larval penetrations 10–14 days after inoculation. After 6-8 weeks, roots of the remaining plants were examined for presence of female nematodes. Forty-five plant species representing 28 genera and 9 families were tested.

Neither penetration of the roots nor reproduction of *H. glycines* occurred on *Narcissus pseudonarcissus* L., *Beta vulgaris* L., *Spinacia oleracea* L., *Lactuca sativa* L., *Brassica oleracea* var. *acephala* DC., *B. oleracea* var. *italica* Plenck, *B. oleracea* var. *juncea* Cass., *B. rapa* L., *Raphanus sativus* L., *Cucurbita pepo* L., *Cucumis sativus* L., *Gladiolus* sp., *Avena sativa* L., *Zea mays* L., *Triticum vulgare* Vill., *Capsicum frutescens* L., *Lycopersicum esculentum* L., *Nicotiana tabacum* L., *Arachis hypogea* L., *Stizolobium deeringianum* Bort., *Trifolium pratense L.*, *Vigna sinensis* (Torner) Savi.

In 14 species, an occasional nematode penetrated the roots but did not reproduce. These species were *B. oleracea* var. *capitata* DC., *Crotalaria mucronata* Desv., *C. spectabilis* Roth, *Lupinus angustifolius* L., *Medicago sativa L.*, *Phaseolus lunatus* L., *Pisum sativum* L., *Psophocarpus tetragonolobus* (L.) DC., *T. incarnatum* L., *T. repens* L., *Vicia angustifolia* L., *V. cracca* L., *V. sativa* L., *V. dasycarpa* Ten. Only slight enlargement of the larvae was noted except that the larvae from *P. lunatus* were considerably enlarged although no molt was observed.

Species on which *H. glycines* reproduced were restricted to the *Leguminosae*. The list of species on which reproduction occurred, with new hosts indicated by an asterisk, is as follows: *G. max*, *G. gracilis* Skvortz*, *G. ussurinesis* Regel & Maack, *P. angularis* Willd., *P. vulgaris* L., *V. villosa* Roth*, *Lespedeza stipulacea* Maxim.*, *L. striata* (Thunb.) H.&A.*, and *L. cuneata* (Dumont) G. Don*.

More cysts were recovered from soybeans than from any other species tested.

AIR DRYING.—Gladiolus corms and narcissus bulbs are grown in the area infested by *Heterodera glycines* in North Carolina. Under the present North Carolina Department of Agriculture quarantine, corms and bulbs air cured for at least 60 days must be thoroughly cleaned of soil and of outer scales prior to shipment from the infested area. At 2 locations, soil was collected from air-cured narcissus bulbs during the cleaning process and examined for the presence of cysts. A few cysts but no viable larvae were recovered. A series of experiments was undertaken to determine if air curing had any effect on the viability of eggs and larvae in cysts. Infested soil was gathered at various times of the year and the cysts screened out and divided into 2 lots. One sample was placed in Baermann funnels, and larvae recovered were counted after 2–3 weeks. The second sample was placed in Syracuse watch glasses and air dried for varying intervals at room temperature (25°–35°C) in a partially closed cabinet. Very few larvae emerged from the air-dried cysts (Table 1).

LIFE HISTORY.—Life history studies were initiated to determine the time required to complete the life cycle of *Heterodera glycines* and to obtain data on the potential increase of the nematode during 1 growing season. Roanoke soybean seedlings growing in 3-in. pots were inoculated with second-stage larvae. After 48 hours these seedlings were removed, the roots were washed, and the seedlings were replanted in fumigated soil. Seedlings were removed subsequently at 48-hour intervals. The roots were examined for the presence of female nematodes, and the soil was examined for the presence of males and second-stage larvae. The experiments were conducted at 23°C.

The second molt occurred 5 days after the roots were inoculated. The third molt was discerned within 8 days, and the fourth molt for the male occurred after 10 days. The fourth molt of the female was not observed. Adult males were found in the soil approximately 14 days after inoculation. The largest number of males was recovered in 20–22 days. Very few, if any, males were collected 35 days after inoculation. Second-generation larvae were found within 21 days. In 1 experiment, approximately 9680 second-generation larvae per pot were recovered 28 days after inoculation. The number of males and larvae recovered varied among experiments.

In an additional test, single egg-masses were used to inoculate individual soybean plants to determine the potential increase of this nematode during a period of 2 months. The number of cysts and mature females recovered from these cultures varied from 280 to 1157.

RELATION OF CYST NUMBERS AND SYMPTOMS ON SOYBEAN.—Pathogenicity tests were conducted in the greenhouse. Soil in 8-in. clay pots was infested with 350, 700, or 1050 cysts, and observations were made on plant growth. Biloxi soybeans were planted. When 350 cysts were used as inoculum, the plants were stunted slightly; however, little or no chlorosis was observed. When 700 and 1050 cysts were used, the plants were severely stunted and the leaves were chlorotic

TABLE 1.—*Larval emergence from cysts of Heterodera glycines air dried at room temperature for various intervals*

Date and source of cyst collection	Days air dried	Number of larvae emerged: Original sample at time of collection	Number of larvae emerged: Air-dried sample
April, field	71	399	19
May, greenhouse	70	44,158	70
June, field	365	2,704	2
August, field	42	52,314	42
August, field	30	6,093	2

FIG. 1. Effects of population levels on plant growth. Pots from left to right were infested with 0, 1050, 700, and 300 cysts of *Heterodera glycines*.

(Fig. 1). Soil samples were collected from infested fields in which soybeans were stunted and chlorotic like those in the previously described greenhouse tests. Cyst counts ranged from 280 to 800 cysts per pint of soil. Thus, a correlation between population levels and symptoms, both greenhouse and field, was noted.

DISCUSSION.—Soybean root leachates were ineffective in stimulating larval emergence from cysts and egg masses of *Heterodera glycines;* Ichinohe obtained similar results (10). Root leachates have also been ineffective in stimulating larval emergence of *H. major* Schmidt 1930 and *H. göttingiana* (2). Apparently, variation occurs in cyst nematodes in relation to the stimulatory effect of root leachates, since leachings from beets and potato roots have been shown to stimulate the hatching of eggs of *H. schachtii* and of *H. rostochiensis* Wr. 1923, respectively (2).

H. glycines has certain hosts in common with other species of *Heterodera*. *H. trifolii* reproduces on *Lespedeza stipulacea* and *Phaseolus vulgaris* (2), *Vicia villosa* (4), and *Glycine max* (14). *H. schachtii* has been reported to reproduce on *P. vulgaris* and *H. göttingiana* on *V. villosa* and *G. max* (2). This group of cyst nematodes appears to be closely related on the basis of host range; however, distinct morphological characters have been reported (6).

Ichinohe (9) reported that a minimum of 24 days was needed for the completion of a generation with this nematode; however, under the conditions of these tests, second-stage larvae were observed 21 days after inoculation of soybean plants. Eggs formed during the current season are capable of hatching immediately, and the population of this nematode can increase rapidly within a growing season under North Carolina growing conditions. It is estimated that 4 or 5 generations per year could develop.

Air-drying experiments showed that desiccation markedly reduced the numbers of viable larvae but did not completely eradicate this nematode. Since bulbs and corms from the infested area are air cured for 60 days or more and thoroughly cleaned prior to shipment, dissemination of this nematode by this means would be unlikely. As an additional safeguard to prevent dissemination of this nematode as a contaminant on bulbs, it is recommended that bulbs grown in infested fields be dipped in a solution of sodium trichlorophenate (14).

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OBSERVATIONS ON THE INFLUENCE OF WEATHER CONDITIONS UPON SEVERITY OF SOME DISEASES OF ALFALFA AND RED CLOVER [1]

D. A. Roberts

SUMMARY

Black stem of alfalfa and northern anthracnose of red clover caused relatively heavy losses of first-harvest hay in central New York when rainfall during the preceding autumn had been adequate to insure abundant production of inoculum, when at least 0.01 in. of rain fell during each of 18 days in May, and when mean temperatures were near or above normal during March, April, and May. These diseases caused relatively little crop loss if there had been no more than 12 days in May with measurable rainfall, even when other conditions favored disease development. When mean temperatures were not higher than 28°, 41°, and 51°F in March, April, and May, respectively, the Pseudopeziza leafspots of both alfalfa and red clover were particularly mild, and none of the 4 diseases studied was very destructive.

Reliable estimates of the relative economic importance of certain diseases of forage crops have been made (2, 3, 4, 7, 8), even though it was not always possible to determine accurately the actual amounts of crops lost. Knowledge of relative severities of diseases, however, is useful in determining the influence of weather upon disease development. Although much is known about environmental factors that favor development of many forage crop diseases under controlled conditions or under field conditions during 1 season, there is little information on the influence of weather on severity of forage crop diseases in the field during several consecutive years. Some correlations between severity of forage crop diseases and weather have been noted, however. Hanson (3) observed that, over a 7-year period, black stem of alfalfa was severe in Wisconsin during the 3 years when spring weather was both cooler and wetter than usual. Similar observations made in other areas (1, 5) indicate that weather conditions occurring as long as a month or more before the first harvest of hay might so affect severity of certain forage crop diseases that reliable disease forecasts could be made.

The study reported here was made to determine the influence of some weather conditions on severity of 4 important diseases of foliage and stems of alfalfa and red clover in New York and to test the hypothesis that severity of these diseases can be predicted with reasonable accuracy.

METHODS.—The 4 diseases studied were black stem of alfalfa (*Medicago sativa* L.), caused by *Phoma herbarum* var. *medicaginis* West. ex Rab. (9) [= *Ascochyta imperfecta* Pk.]; leafspot of alfalfa, caused by *Pseudopeziza medicaginis* (Lib.) Sacc.; northern anthracnose of red clover (*Trifolium pratense* L.), caused by *Kabatiella caulivora* (Kirch.) Karak.; and leafspot of red clover, caused by *Pseudopeziza trifolii* (Fr.) Fckl.

From 1952 to 1956, severity of each of these diseases was estimated in New York meadows. In each field, at least 10 plants in each of 3 widely separated areas were examined for foliage and stem diseases. Disease severity was rated after diseased foliage had been compared with leaflet diagrams on which measured areas had been marked to represent necroses. Data were recorded for percentage of plants affected and for severity of each disease on the lowest, center, and top thirds of plants. After leaf/stem ratios for cured hay of each crop had been determined, it was possible to calculate approximate hay losses due to each disease (8). In a given field, there was close agreement between ratings made by 1 observer on different days and between any 2 ratings made simultaneously by several observers.

The diseases studied were most destructive to leaves on the lowest one-third of affected plants. These leaves account for only about 10 per cent of the weight of cured alfalfa or red clover hay, and such leaves invariably are attacked by several pathogens. Consequently, estimated percentages of hay lost to any 1 disease were low. Even though such estimates might be in error as far as absolute values are concerned, they are meaningful for purposes of ranking crop losses due to several diseases during any year or of determining relative values of losses due to 1 disease during several years.

Rainfall and temperature conditions in the autumn and spring preceding harvest are the environmental factors that most strongly influence severity of the 4 diseases studied (1, 4, 5, 6). The number of days during which there was measurable rainfall and the total rainfall occurring in October and May before harvest were recorded. Mean temperatures during autumns from 1951 to 1955 did not vary sufficiently in any 1 of 3 widely separated areas in New York to have accounted for important differences between years in amounts of overwintering structures of the 4 pathogens studied. Mean temperatures for the months of March, April, and May, however, were variable from year to year and were recorded because they were considered important in the production of inocula for both primary and secondary cycles of diseases.

Although disease severity data were taken in all parts of New York, most observations were made in the central region, which consists of counties between the

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Assistance of K. D. Fezer, R. T. Sherwood, C. S. Ramamurthi, and C. H. Ward in making disease surveys is gratefully acknowledged.

Finger Lakes and the Hudson River, south of an eastward projection of the southern shore line of Lake Ontario. Disease-severity data from this area were compared with rainfall and temperature data from the Ithaca weather station. These data were as representative for the region as were data recorded at any other station.

RESULTS AND DISCUSSION.—Black stem of alfalfa and northern anthracnose of red clover were more severe in 1953 than in any other year (Table 1), and the number of days with measurable rainfall during the preceding October and May had been 17 and 18, respectively. Apparently the number of days during which there was measurable rainfall, both in October and in May before the harvest, was more important than was total rainfall as far as severity of either of these 2 diseases was concerned. Neither disease became epiphytotic, regardless of total rainfall, unless there had been at least 18 days with measurable rainfall during the May before harvest. Further, when the number of rainy days in May did not limit disease development, as in 1952 and 1956, the number of rainy days during the October before harvest was more nearly correlated with disease severity than was total rainfall. Rainy weather in October preceding harvest would favor infections that would provide an abundance of overwintering inocula, since both *Phoma herbarum* var. *medicaginis* and *Kabatiella caulivora* survive winters on diseased stems and leaves (1, 4). The relatively little damage done by black stem of alfalfa in 1952 probably was attributable to the scarcity of rainy days in October of 1951, since there were 20 days with measurable rainfall in May of 1952.

Pycnidia of the alfalfa black stem fungus do not form abundantly in the spring on infected stems and leaves (1, 5), and most infections at that time of year probably are initiated by primary inocula from plant debris. Hence, during every period of wetting, conidia are likely to be extruded from pycnidia and disseminated to infection courts. The other 3 diseases studied develop in the spring as a consequence of secondary cycles of disease (4, 6); although periods of wetting favor production and dissemination of secondary inocula, frequent wettings probably are not so important for development of northern anthracnose and the Pseudopeziza leafspots as they are for development of alfalfa black stem.

Pseudopeziza leafspots of both alfalfa and red clover caused about the same amounts of crop losses each year between 1953 and 1955 (Table 1) in spite of the fact that there were approximately twice as many rainy days in the months of October 1952 and May 1953 prior to harvest as there were in the same months before the 1954 and 1955 harvests. Both leafspot diseases were unusually mild in 1956, when mean temperatures for March, April, and May were much lower than they had been during the 5 preceding years. Between 1951 and 1955, weather conditions in autumn apparently had no important influence upon amounts of primary inocula of *Pseudopeziza medicaginis* and of *P. trifolii* (6) available for infections each spring. Further, there was sufficient rainfall every spring to account for production and dissemination of secondary inocula. Consequently, the Pseudopeziza leafspots developed to approximately the same degree of severity each year, with the exceptions that neither disease caused more than a small amount of loss in 1956 and that the Pseudopeziza leafspot of red clover was considerably less severe than the Pseudopeziza leafspot of alfalfa in 1952. Probably the unusually cold weather during March, April, and May of 1956 limited the rates of occurrence of secondary cycles of both diseases during that year. The difference in severity between the 2 diseases in 1952 is difficult to explain. It was noted, however, that target spot of red clover, caused by *Stemphylium sarcinaeforme* (Cav.) Wilt., was more severe in 1952 than in any of the other years. Possibly, target spot developed so rapidly and became so severe in 1952 that it destroyed leaf tissues that might otherwise have served as infection courts for *P. trifolii*. No pathogen such as the target spot fungus competed with *P. medicaginis* on alfalfa.

The correlations observed between weather conditions and severity of black stem of alfalfa and northern

TABLE 1.—*Estimated percentages of hay losses due to black stem and Pseudopeziza leafspot of alfalfa and to northern anthracnose and Pseudopeziza leafspot of red clover in central New York, 1952-1956, inclusive, and rainfall and temperature data recorded at Ithaca, New York,[a] from 1951 to 1956*

	Percentages of hay losses				Weather data						
	Alfalfa		Red clover		Rainfall						
					Total in.		No. days[c]		Mean temperature (°F)		
Year[b]	Black stem	Pseudopeziza leafspot	Northern anthracnose	Pseudopeziza leafspot	Oct.	May	Oct.	May	March	April	May
1951-52	2.5	3.1	1.2	1.5	1.0	4.7	8	20	32	48	53
1952-53	5.1	3.0	6.0	3.0	3.1	3.4	17	18	36	44	58
1953-54	1.0	3.0	1.5	3.0	1.5	3.3	10	7	33	47	55
1954-55	1.0	3.0	2.0	3.0	1.6	1.7	6	7	35	50	58
1955-56	2.3	0.4	1.3	0.2	8.2	1.9	9	12	28	41	51

[a] Figures obtained from *Climatological Data, New York*, published by the Weather Bureau, U.S. Department of Commerce.
[b] The crop season is represented by the second of the 2 years recorded on each line.
[c] Number of days with at least 0.01 in. of rainfall.

anthracnose of red clover provide evidence that these 2 diseases are likely to become epiphytotic before the first harvest of hay in central New York when there are approximately 20 days in May with measurable rainfall, provided rainfall in autumn and mean temperatures for March, April, and May had been near normal. Further, these diseases probably will be particularly mild if there are no more than 10 days in May with measurable rainfall. The Pseudopeziza leafspots of alfalfa and red clover are likely to be particularly mild in central New York when average mean temperatures for March, April, and May are not more than 28°, 41°, and 51°F, respectively. These diseases probably will be moderately severe, however, when mean temperatures for March, April, and May are near the long-term average values of 34°, 46°, and 57°F, respectively.

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PHYTOPATHOLOGICAL NOTES

Maintenance of Contaminant-free Stain Preparations for the Detection of Bacteria in Plant Tissues.[1] W. A. F. Hagborg[2]. The presence or absence of bacteria in plant tissues is conveniently and effectively detected by microscopic examination of a negatively stained smear preparation. The method described by Burkholder[3] has been preferred in this laboratory, but it was necessary to modify the stain formulation several years ago, because the aqueous solution of congo red became contaminated with rod-shaped bacteria at room temperatures. The removal of such cells by Seitz filtration proved to be of only temporary effectiveness, but the substitution of 0.1 per cent mercuric chloride for distilled water in the staining solution prevented contamination indefinitely without reducing the effectiveness of the stain. Stain coagulation, which occurred with the more acid host tissues, could be avoided usually by a further modification, i.e. alkalization of the smear with sodium bicarbonate solution before application of the stain.

In the modified staining procedure, a small portion of the lesion is chopped on a clean slide in a drop of an aqueous solution containing 0.1 per cent mercuric chloride and 2 per cent congo red. After diffusion, the coarser solids are scraped from the slide. The remaining stain is spread in a thin film and allowed to dry without application of heat. A few drops of acid alcohol are run over the film to change the color of the dye to a deep blue. As soon as dry, the film is ready for microscopic examination.—Plant Pathology Laboratory, Science Service, Canada Department of Agriculture, Winnipeg.

[1] Contribution No. 1602 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
[2] Plant Pathologist, Winnipeg.
[3] Burkholder, W. H., 1937. A bacterial leaf spot of geranium. Phytopathology 27: 554-560.

A New Spore Form in Rhynchosporium secalis.[1] W. P. Skoropad and A. H. H. Grinchenko.[2] The first formal description of the fungus now known as *Rhynchosporium secalis* (Oud.) Davis was provided by Saccardo.[3] His description as well as that of Heinsen[4] or that of Davis[5] referred to 1 spore form only. This was the uniseptate, beaked, hyaline conidium that initiated the scald disease of some cereals and grasses. Present investigations of the fungus have revealed the presence of another spore form, and the purpose of this paper is to report on some morphological and cytological features of this form, which at present will be referred to as a microconidium.

Most of the material for the study was cultured on thin films of nutrient agar on microscope slides, and

[1] Contribution No. 1604 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.
[2] Associate Plant Pathologist and Assistant Technician, respectively.
[3] Saccardo, P. A. 1906. Sylloge fungorum 18: 540.
[4] Heinsen, E. 1901. Beobachtungen über den neuen Getreidepilz Rhynchosporium graminicola. Jahrb. Hamburg. Wiss. Anstalt. 18: 43-55.
[5] Davis, J. J. 1919. VI. Notes on parasitic fungi in Wisconsin. Trans. Wisconsin Acad. Sci. 19: 705-727.

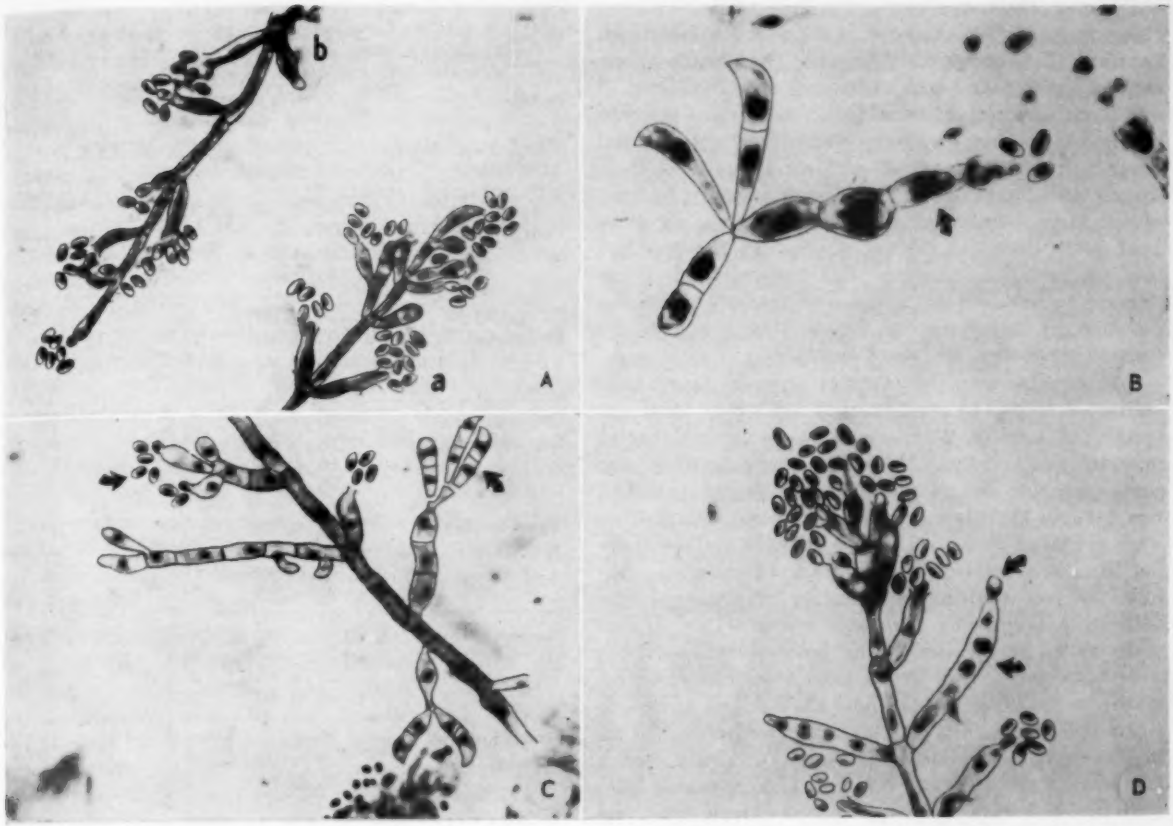

FIG. 1. Morphological and cytological features of microconidia of *Rhychosporium secalis* and of the branches that bear them. A) Arrangement of microconidial branches on mycelial strands, showing opposite positions (a) and alternate positions (b). B) Microconidial branch arising directly from conidium. C) Conidia and microconidia produced on the same hypha. D) Position of nuclei in microconidial branch and emergence of first microconidium.

nuclear phenomena were investigated with the aid of HCl-Giemsa stain.[6]

The microconidia are globose to oblong, 2.5–7.5 × 1.5–2.5 μ, and hyaline. Each contains a single nucleus that is cup-shaped and occupies about two-thirds of the volume of the spore. They are produced within 1-celled flask-like branches of the mycelium. The branches usually occur opposite one another, but they also may be arranged alternately (Fig. 1, A, C). In rare cases a branch arises directly from a conidium (Fig. 1, B). A single hypha or conidium may bear both true conidia and the branches that produce microconidia (Fig. 1, C, B).

The nucleus in each branch apparently divides mitotically so that several of the daughter nuclei may appear in line (Fig. 1, D). Cell walls form around these nuclei, and the first microconidium moves into the branch tip. The branch tip ruptures and the microconidium is ejected through the aperture (Fig. 1, D). The formation of aggregations of microconidia at some distance from the ruptured branch tip suggests that the ejection is accomplished with some force. Single branches have been observed to produce from 2 to 24 microconidia, and often these branches are then devoid of nuclei, which indicates that production has ceased.

Microconidia are produced in a variety of common nutrient-agar media and over a wide range of temperatures, although they occur most abundantly at 10–18°C. They are not formed on young mycelium but only on older mycelium that already has produced conidial clusters.

The function of these microconidia is still a matter of speculation. Attempts to germinate them have failed, and they have not been observed to fuse with any other structures in the culture. There also has been no evidence that they change into other spore forms. On the basis of the occurrence of morphologically similar microconidia in another genus,[7] however, it is reasonable to suppose that they may be functionless male cells or that their sexual role could be revealed if the perfect stage of *R. secalis* were known.—Plant Pathology Laboratory, Science Service, Edmonton, Alberta.

[6] Robinow, C. F. 1945. Addendum: Nuclear apparatus and cell structure of rod-shaped bacteria. In R. J. Dubos, ed., The bacterial cell. Harvard Univ. Press, Cambridge, Mass. p. 355-377.

[7] Drayton, F. L. 1934. The sexual mechanism of Sclerotinia gladioli. Mycologia 26: 46-72.

Effect of Antibiotics on the Numbers of Bacteria and Fungi Isolated From Soil by the Dilution-Plate Method. LEANDER F. JOHNSON. Although the dilution-plate method for isolating and estimating the population of soil fungi has certain limitations, the method is commonly used. One disadvantage is that not all soil fungi will grow on any 1 medium because of their diverse nutritional requirements. Furthermore, because of the large number of bacteria that occur in soils, 1 or more bacterial inhibitors must be incorporated into the isolation medium. The present work is an attempt to improve the dilution-plate method for the isolation of soil fungi by determining the effect of various antibiotics on colony counts.

Soil samples were collected at different times from cultivated fields. In most cases, a fine sandy loam was used. The final dilution of soil (on a dry-soil basis) to water was 1:10,000. Martin's peptone-dextrose agar containing rose bengal at a concentration of 1:30,000[1] was used as the plating medium. Antibiotic solutions were prepared by adding sterile water to vials containing the sterile crystalline or powdered material. Aliquots of the resulting solutions or suspensions were withdrawn aseptically and diluted with water, so that when added to the media, the desired concentrations were obtained. These sterile antibiotic solutions were added to flasks of the medium after it had cooled to just above the solidifying point. Approximately 15 ml of the media was poured into each Petri dish, which contained 1 ml of the soil dilution. Colony counts from 5 Petri dishes were made after 5 days of incubation and averaged for each concentration of the antibiotics tested.

When added to the above medium, streptomycin at 30 μg/ml, as recommended by Martin[1], inhibited bacterial colonies effectively (Table 1). Of 22 soil dilutions made, an average of 0.8 bacterial colonies per plate developed when streptomycin was used. The antibiotics most effective in inhibiting soil bacteria at low concentrations were the polycyclines, namely tetracycline, chlortetracycline, and oxytetracycline. They compared favorably with streptomycin in this respect. In additional tests, significantly fewer bacterial colonies developed on media containing 2 or 5 μg of chlortetracycline than on media containing streptomycin. The few bacteria that did develop at these concentrations were pin-point colonies. Significantly more fungal colonies developed on media containing 2, 0.5, or 0.25 μg of chlortetracycline than on media containing streptomycin. Although the difference in the number of fungal colonies per plate was small, this would usually represent a large difference in estimates of the number of fungi in soils. In this case, it represented differences in estimated fungal populations of 17,000 and 38,000 per gram of soil at 2 and 0.5 μg/ml of chlortetracycline, respectively.

An explanation as to why more fungi were obtained on soil-dilution plates containing chlortetracycline than on those containing streptomycin can be only speculative at this time. Martin[1] reported that streptomycin retarded the growth of a few fungi in pure culture. Warcup[2] found that in "soil plates" some fungi developed after 2 weeks of incubation on Czapek-Dox agar acidified with phosphoric acid. In the present study, fast-growing fungi were present in most plates, and this prevented counting after 1 week of incubation.

TABLE 1.—*Numbers of soil microorganisms that developed on soil-dilution plates when streptomycin and 8 other antibiotics were added singly to Martin's peptone-dextrose agar containing rose bengal*

Antibiotic	Concentration	Average no. of colonies per plate[a]	
		Bacteria	Fungi
Streptomycin	30 μg/ml	0.7	12.0
Penicillin G	100 units	2.8*	9.0*
	50 units	6.0**	10.7
	25 units	12.8**	10.0
Streptomycin	30 μg/ml	0.4	11.6
Polymyxin B	100 μg/ml	0.8	6.0**
	50 μg/ml	3.0**	9.6
	25 μg/ml	2.7**	10.4
Streptomycin	30 μg/ml	0.4	10.8
Erythromycin	100 μg/ml	3.6*	12.2
	50 μg/ml	6.5**	11.0
	25 μg/ml	6.5**	13.0
Streptomycin	30 μg/ml	1.2	10.4
Neomycin	100 μg/ml	2.8*	12.2
	50 μg/ml	12.0**	12.5
	25 μg/ml	20.5**	8.8
Streptomycin	30 μg/ml	3.0	16.0
Chloramphenicol	50 μg/ml	4.6*	16.2
	25 μg/ml	24.0**	14.0
	10 μg/ml	21.2**	15.7
Streptomycin	30 μg/ml	0.4	11.0
Tetracycline	20 μg/ml	0.2	9.4
	10 μg/ml	0.4	12.2
	5 μg/ml	0.2	9.6
Streptomycin	30 μg/ml	0.4	9.0
Oxytetracycline	5 μg/ml	0.4	9.8
	2 μg/ml	0.4	9.4
	1 μg/ml	0.6	9.0
Streptomycin	30 μg/ml	0.3	11.4
Chlortetracycline	5 μg/ml	0.0**	11.9
Streptomycin	30 μg/ml	0.6	10.1
Chlortetracycline	2 μg/ml	0.1**	11.8**
Streptomycin	30 μg/ml	1.3	10.0
Chlortetracycline	0.5 μg/ml	2.2	13.8**
Streptomycin	30 μg/ml	0.8	11.5
Chlortetracycline	0.25 μg/ml	5.4**	15.3**

[a] Each figure is the average number in 5 plates after 5 days of incubation, except that each figure for chlortetracycline is the average number in 15 plates in 3 separate experiments. Single and double asterisks indicate that the difference between the indicated count and that for the corresponding plates containing streptomycin is statistically significant at the 5 or 1 per cent level, respectively.

[1] Martin, J. P. 1950. Use of acid, rose bengal, and streptomycin in the plate method of estimating soil fungi. Soil Sci. 69: 215-232.

[2] Warcup, J. H. 1950. The soil-plate method for isolation of fungi from soil. Nature 166: 117-118.

It is postulated that either streptomycin inhibited the development of certain soil fungi during the 5-day period of incubation or that chlortetracycline stimulated the development of certain fungi.

Crystalline Aureomycin buffered with sodium glycinate, a form of chlortetracycline suitable for intravenous injection, was used in this study. Since solutions of Aureomycin tend to lose their potency on standing, fresh solutions should be prepared at the time they are to be used. When soil dilutions are to be made over a period of several days, however, sterile solutions made with distilled water containing not more than 1 mg/ml may be dispensed in screw-cap tubes or vials and stored in the frozen state. Care should be taken to avoid alkaline or neutral solutions, since Aureomycin is unstable at or above pH 7.0. For each experiment, only as many tubes as are needed should be thawed and mixed thoroughly. Any unused portion of the solution should be discarded. Solutions stored in this manner may be used with assurance for periods up to 2 months.[3]—Department of Plant Pathology, Agricultural Experiment Station, University of Tennessee, Knoxville, Tennessee.

Chromatographic Study of Amino Acid Development in Wheat Ovules in Relation to Genes for Disease Resistance. F. P. Zscheile and Hazel C. Murray. Griffith, Zscheile, and Oswald[1] demonstrated that environmental conditions, especially temperature, can influence greatly the effective resistance or susceptibility of Baart wheat to bunt, caused by *Tilletia caries* (DC.) Tul. Following a period of germination and growth at 10°C, 100 per cent of the plants of this variety may become bunted. The isogenic partner, Baart 38, has bunt resistance conferred by the Martin gene; when grown under these same circumstances, Baart 38 may not be visibly bunted but a small number of chlamydospores of the bunt fungus may be found in the kernels by microscopic examination. When grown to maturity at 10°, even Baart 38 will have a considerable proportion of visibly bunted heads. Later, Zscheile[2] induced similar breakdown of bunt resistance in 5 other derived varieties having the Martin gene.

These environmental conditions may alter the resistance of these wheats to bunt by promoting chemical changes within the developing ovule. Such changes may provide the nutritional conditions[2,3] that permit the bunt fungus to complete its life cycle and produce chlamydospores. Steinberg[4] considered amino acids as possible factors in disease resistance. We have been searching for differences between the composition of ovules of resistant wheat and that of ovules of susceptible wheat; comparative results of amino acid studies made by paper chromatography are reported herein.

The varieties studied included 6 pairs, the members of which are isogenic except for the Martin gene. These were Bunyip and Bunyip 41, Escondido and Escondido 41, Federation and Federation 41, Onas and Onas 41, Pacific Bluestem and Pacific Bluestem 37, and Sonora and Sonora 37. Of each pair, the numbered member is a backcross of the first and contains the Martin gene. The number refers to the year of introduction to agricultural use. In 5 other similar pairs (or trios), the bunt-resistant backcrosses (numbered) were also resistant to stem rust. These were Baart, Baart 38, and Baart 46; Big Club and Big Club 43; Poso and Poso 48; Ramona and Ramona 44; and White Federation and White Federation 38. Big Club 43 and Poso 48 are also resistant to the Hessian fly.

Young heads of field-grown wheat were picked at 2 stages of development: 1) at anthesis, when pollen was well developed; and 2) after anthesis, when the ovules had a length of 4–5 mm and a weight of 15–20 mg. Fresh ovules were removed individually from their hulls. A 2-g sample was cytolyzed with ether, ground in a mortar with 2 ml of 95 per cent ethanol, and centrifuged; the supernatant was removed and made to 2 ml. Such extracts of free amino acids from 28 varieties were compared by 2-dimensional paper chromatography for content of 16 amino acids and of ethanolamine. Solvents were mixtures of butanol, acetic acid, and water (4:1:5 v/v/v) and of phenol and water (4:1 v/v), used on Whatman No. 1 chromatograms 18 × 22 in. in size.

Only relative amounts of each amino acid were estimated by visual comparison of color intensities and sizes of the spots developed by the ninhydrin reagent. Known quantities of standard amino acids were studied under identical conditions to provide correction factors for differences in color development. Amounts found were rated numerically for comparative purposes, numbers above 10 representing notably large and intensely colored spots.

Ovules of susceptible and derived resistant varieties differed appreciably in average content of ethanolamine, lysine, and leucine at anthesis and in average tyrosine content after anthesis (Table 1). The averages for all other amino acids were very similar for resistant and susceptible varieties. The relative ratings and the order of abundance were not the same for all groups; however, most amino acids changed but little in rank at the 2 growth stages considered. Three amino acids changed considerably in relative abundance; lysine and tyrosine decreased after anthesis whereas phenylalanine increased.

The early work of Blish and Olson[5] indicated that

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TABLE 1.—*Relative abundance of various amino acids in ovules of 11 bunt-susceptible wheat varieties and of 12 derived bunt-resistant varieties*

	Susceptible varieties				Resistant varieties			
	At anthesis		After anthesis		At anthesis		After anthesis	
Amino compound	Average rating [a]	Rank	Average rating	Rank	Average rating	Rank	Average rating	Rank
α-Alanine	6.5	5	6.8	4	6.3	5	6.1	5
Arginine	6.0	7	4.0	6	5.7	6	3.8	8
Asparagine	17.4	1	12.0	2	16.2	1	13.6	1
Aspartic acid	1.5	15	1.6	13	1.5	13	2.2	12
Ethanolamine	2.6	13	1.8	12	1.8	12	2.0	13
γ-Amino-butyric acid	8.8	4	8.9	3	8.8	4	8.7	4
Glutamic acid	11.4	3	8.9	3	10.7	3	9.8	3
Glutamine	13.5	2	12.4	1	12.9	2	12.2	2
Glycine	3.0	12	2.9	10	3.0	11	3.2	10
Leucine	5.9	8	4.4	5	4.5	9	5.0	6
Lysine	6.3	6	3.0	9	5.2	7	3.0	11
Phenylalanine	1.4	16	2.1	11	1.4	14	1.9	14
Proline	4.9	10	3.6	8	5.0	8	3.4	9
Serine	5.7	9	3.8	7	5.0	8	4.5	7
Threonine	1.1	17	1.1	15	1.4	14	1.2	15
Tyrosine	1.8	14	1.3	14	1.4	14	0.3	16
Valine	3.8	11	3.0	9	3.5	10	3.0	11

[a] The numerical values indicate relative abundance of compounds and have an estimated error of ±1 unit. Values above 10 indicate large and intensely colored spots.

formation of storage protein in the wheat ovule does not occur until desiccation begins and that the amino acids are in the free form at the early stages. Blish's amino acid analyses[6] of immature kernels are at some variance with those of ovules reported here.

Miller et al[7] found only minor differences among wheat varieties in contents of lysine and glutamic acids. Our results on glutamic acid were similar to theirs, but we found extreme variation in lysine contents. The downward trend (with time) that we noted in lysine content is in the right direction to support their finding of 10 times as much glutamic acid as lysine in mature grain. McElroy et al[8] reported more phenylalanine than arginine in mature Marquis wheat protein hydrolysates, which is in agreement with the upward trend of phenylalanine indicated here. Pence and co-workers[9] reported uniformity of amino acid contents of glutens in mature grain of 17 varieties of wheat of many types and sources. They found approximately 10 times as much glutamic acid as aspartic acid and high contents of proline. These differ from our results with ovules and probably indicate changes occurring later in kernel development.

The relative abundance of individual amino acids was as follows: among neutral amino acids—γ-amino butyric acid> α-alanine> leucine> serine>valine = glycine> threonine; among acidic amino acids—glutamic acid> aspartic acid; among basic amino acids—asparagine> glutamine> arginine> lysine; among aromatic amino acids— proline> phenylalanine> tyrosine (low). After anthesis, decreases were recorded in some varieties for the following amino acids: leucine, serine, glutamic acid, arginine, asparagine, lysine, and tyrosine.

Other varieties, with their genes for bunt resistance in parenthesis, were Martin (M), Oro (T), Rio (R), Selection 1403 (H), and Turkey 3055 (T). These are not reported in Table 1.

Specific details on certain varieties follow: Martin is high in glycine, leucine, valine, arginine, tyrosine, and proline and moderate in glutamine; Rio is high in glutamine and Turkey 3055 in asparagine. Selection 1403 is high in serine after anthesis. Escondido, Onas, Sonora, and White Federation are low in aspartic acid, whereas Bunyip, Escondido, and White Federation are low in glycine. Martin, Rio, Oro, and Ramona are high in proline, Bunyip and Ramona are low in threonine, and White Federation is low in asparagine, lysine, and tyrosine.

On the genetic side, we may make certain tentative conclusions. The Martin gene for bunt resistance and the Hope gene for rust resistance do not appear to influence greatly the amino acid patterns of the ovules of derived disease-resistant varieties. Likewise, the gene for Hessian fly resistance has no apparent effect on the amino acid pattern. These data indicate that resistance or susceptibility to the pests considered do not appear related to the free amino acid patterns of wheat ovules. —Department of Agronomy, University of California, Davis, California.

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